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LABORATORY METHODS

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LABORATORY METHODS

WITH SPECIAL REFERENCE TO THE NEEDS OF

THE GENERAL PRACTITIONER

BY

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THIRD EDITION

ILLUSTRATED WITH FORTY-THREE ENGRAVINGS

ST. LOUIS

C. V. MOSBY COMPANY

1915

156765.
21.10.20.

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Press of
C. V. Mosby Company
St. Louis

TO
THE GENERAL PRACTITIONER,
WHO MUST BE A SPECIALIST IN ALL BRANCHES OF MEDICINE,
THIS BOOK IS DEDICATED
BY THE AUTHORS.



PREFATORY NOTE

A realization of the fact that the general practitioner is not usually prepared to make, on account of lack of extensive apparatus and other conveniences, elaborate chemical tests and examinations, has prompted the authors to prepare this book. "Laboratory Methods" is not of an encyclopedic form, nor is it a limited compend, and is especially designed for the general practitioner who desires to make, easily and inexpensively, examinations on which he may depend.

The physician may have experienced some discouragement in attempts to conduct certain examinations, but he has probably been confused by the complexity of the large book and thwarted by the paucity of the compend. It is not presumed that the practitioner shall attempt every investigation, but this book will show that many of the comparatively simple cases that are usually sent to distant cities for expert examination may be made with more satisfactory results by the practitioner.

It has been the aim of the authors to simplify methods both as to apparatus and technic. Essential factors have not, however, been omitted, but have been emphasized in such manner as to indicate their importance. Only the best tests are given, so that the reader will not be perplexed by being obliged to do any choosing for specific cases. Stress has been laid on safe diagnosis, and sources of error, as well as the value and limitation of tests, have been pointed out.

B. G. R. W., M. D.

E. G. C. W., M. D.

PREFACE TO THIRD EDITION.

It is the aim of the authors to keep this little volume true to name and purpose. This cannot be realized if extensive alterations are made in each edition. The simple methods suggested have been used for many years and have proven a right to a permanent place. We are not justified in substituting newer methods until they prove themselves superior to the older ones.

Some procedures have been added to the text, but we have preferred to place the majority of these in the appendix rather than disturb the simplicity of the text. Among the chief of these are Elastic Tissue Staining, Salting-Out Method for Tubercle Bacilli, A Simple Gram Stain, An Invariable Blood Stain, A Dressing for Laboratory Tables, and a number of other notes.

Since making the second revision, a thousand and one laboratory innovations have appeared in literature. Many have been tried out and abandoned, others must be tested more thoroughly before being included in texts, still others are too difficult to find a place in a book designed for the practitioner and a great many are not worth trying out.

The authors realize that no book of this size can include all that is good in the medical laboratory, and recommend frequent reference to the larger texts.

B. G. R. W., M. D.

E. G. C. W., M. D.

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INTRODUCTION.

BY VICTOR C. VAUGHAN, M. D.

It gives me great pleasure to write a short note of approval of this book. It has been said that the general practitioner is passing away, but this statement is certainly negated by this book. There is nothing more hopeful in the practice of medicine today than the thorough way in which many general practitioners are doing their work. There are many small cities, and even villages, in which there are general practitioners who have equipped themselves with most effective laboratories. This volume shows that the working laboratory in which the best work may be done can be established at a small cost. It requires only a good man to conduct it. It would be regrettable were it true that the country doctor has ceased to do scientific work. Jenner was a village doctor when he tested and demonstrated the efficiency of vaccination for smallpox. Pasteur had shown himself a great scientist before he ever saw Paris. Koch was a stabsarzt, remote from any great medical center, when he devised solid culture media for the growth of bacteria, and opened up a method of scientific investigation which has given such brilliant results. Sims was a practitioner in the then village of Montgomery, Alabama, when he worked out the technic of the successful operation for vesico-vaginal fistula. Long was a rural doctor in Georgia when he first removed a tumor under general anesthesia. Pollender was a country doctor when he first studied the blood of animals sick with anthrax, and demonstrated rod-like organisms in the same. Beaumont was an army surgeon, stationed at an isolated post on the Island of Mackinac, in the then territory of Michigan, when he made his now classical experiments upon Alexis St. Martin. Indeed, if we take away from medicine the contributions to that science made by physicians far removed from great commercial centers, we rob it of half its glory and its honor.

This little volume shows how the general practitioner can, at a very small cost, equip a laboratory in which he can do most excel-

lent work. It demonstrates that costly apparatus and marble rooms are not necessary for the prosecution of scientific medicine.

It gives me great pleasure, after a careful reading of the proof, to commend most highly this volume.

ANN ARBOR, MICHIGAN.

LABORATORY METHODS.

CHAPTER I.

GENERAL CONSIDERATIONS.

McDowell, who performed the first ovariectomy, had not at his elbow the nickel-plated sterilizer and the gowned assistant, but to his ears came the mutterings of a mob which had sworn to take his life if he failed in the operation. Sims worked and enjoyed his labors under circumstances that would have deterred many physicians, and Beaumont had not the advantage of even an occasional visit to the city clinics.

Medical analyses, meaning those procedures where chemistry and microscopy are used to aid in making correct diagnoses, have gained an important position—too important to be ignored or turned mutely over to the expert.

LABORATORY EQUIPMENT.

When purchasing laboratory equipment, it is advisable to proceed carefully. Too many laboratories, especially those which fail to give satisfactory results, are fitted too hurriedly. While it is not suggested to pay high prices for a popular trademark, some very worthless apparatus is being sold. Stains, as well as solutions for quantitative work, should be purchased in liquid form ready for use, and a number of reputable firms prepare good reagents.

In this book, where possible, the English system of weights and measures has been used.

The authors have listed several departments in order that the various needs of physicians may be met, as it is much better to become expert in blood analyses alone than to conduct incompletely several lines of work.

Microscope.—The microscope, once a luxury, is now imperative for a safe diagnosis. It may be purchased for less than the more elegant examining chairs, and is, to say the least, just as necessary. A good instrument may be had for \$80, this price including the third or oil immersion objective—not a necessity, but certainly a convenience. For ordinary work a movable stage is unnecessary, though many men who have not been forced to work without it would not agree with this conclusion. It is well to remember that Americans make good lenses.

Glassware.—A few cents will buy a stock of glass tubing and stirring rods, and gray filter paper is cheap. A glass funnel and one or two graduates are very convenient.

Centrifuge.—A hand centrifuge, with extra milk tube, may be purchased at a low figure. Sedimentation glasses are possible, though not ideal, substitutes, and when collecting urinary sediments a little thymol should be added to prevent fermentation. Not only does the centrifuge save time, but serves to “bring down” elements which would otherwise remain suspended in the sedimentation glass.

Slides.—Glass slides and covers are obtainable at any physicians’ supply house, and cost very little. For the finer work these slides should not be too thick. On the other hand, a piece of window glass 2x2 inches serves well for urine examinations. Slides which have been used should not be cast aside as worthless. If permanent preparations have not been made, a little alkali solution will remove smears of bacteria or blood. If these have been fixed or stained, the solution may be made stronger. Balsam may be removed by xylol. A dip in grain alcohol may precede washing in water.

Accessories.—The following are some of the laboratory accessories, with prices:

File for glass work.....	.05
Platinum wire	.30
Evaporating dish	.15
Ring stand equipped.....	\$1.00

The selection of rings for the ring stand should include a special clamp to serve as a buret support.

Cleaning Glassware.—Money and time will be saved by cleanliness. For glassware, hot suds followed by hot water are best.

Polish with a soft, dry cloth. Strong mineral acids will remove organic matter.

Stains.—Wright's blood stain, carbol-gentian violet, fuchsin, and methylene blue are necessary for the blood and bacteriological work. For tissues, carbol thionin gives a beautiful effect. Hemalum or alum carmine may be substituted, and then the preparation stained with eosin if a beautiful permanent mount is desired. Alcohol, carbol-xylol, and balsam are necessary only in case the preparation is to be filed. Balsam should not be bought in bottles, but in tubes, in order to prevent waste and decoloration as well as contamination, etc. A paper-filtered balsam is most transparent. A spatula or section lifter may prevent the ruining of many thin sections. It is well to remember that in case the stain should be overturned and spilled, recourse may be had to certain anilin inks. For this purpose eosin, gentian violet, fuchsin, and certain other stains may be obtained in every hamlet, and extraneous matter removed by filtration. Good liquid stains should, however, be used when possible. Certain fabric dyes have been used to demonstrate the gonococcus. India ink and its American substitutes do not stain microorganisms, but have been recommended as a substitute for the high-priced dark field attachments in searching for the *treponema pallidum*.

Substitutes.—Common blotters may be used to take up excess liquids. A brandy bottle makes a good flask unless contents are to be boiled, a procedure rarely or never necessary in clinical analyses. Whisky goblets may be used as beakers when titrating or as wash dishes, while saucers serve as watch glasses. Small new tin ointment boxes, such as are used in dispensing, may be substituted unless acids are used, the bright background forming an excellent contrast to the floating sections. Two-ounce bottles—round or square, to prevent overturning—serve well for liquid reagents. Small salt-mouth bottles or granule bottles are best for solids. Raised glass labels are unnecessary, but glass stoppers, especially for the stronger acids and alkalies, are imperative. It is advisable to keep only a small quantity of the reagent in the bottle which is to be used in order to guard against evaporation, contamination, or spilling. Stock reagents should be kept in a dry basement during the summer months, and never exposed to the light.

Indicators.—A dilute solution of phenolphthalein is the cheap-

est, as well as the best, chemical indicator. When the solution is acid or neutral, it is colorless. Alkalinity results in a red coloration. Litmus paper is too crude for medical work. An aqueous solution of azolitmin is more reliable. An alcoholic solution of the skin of a red radish serves as an excellent indicator. The solution is turned pink by acids and yellow by alkalies.

Cover Glasses.—Round cover glasses are well adapted for smears, but the squares are more convenient for tissue sections. They may be cleaned by immersion in a little alcohol, rinsing in hot water, and polishing with a dry, soft cloth. A "linty rag" should be avoided, as vegetable fibers do not add to the beauty of the microscopic field. It does not follow, however, that in the event such contamination occurs the physician should confuse these with elastic fibers or urinary casts.

Apparatus Cabinet.—An old bookcase will serve well to keep dust off apparatus, as a general house cleaning preliminary to a test should not be necessary. The inside of this case should be painted white and the outside black.

Laboratory Nostrums.—The "general stain-all," the "glass cleaners," and many other preparations offered to the indifferent practitioner may be aptly termed "laboratory nostrums." The secret "blend" and the advanced price seem to be the only distinctions from those efficient formulas well known to all scientific students. The urinary test tablets should be avoided.

Suggestions.—In each chapter helpful suggestions have been made in regard to good, but not expensive, apparatus. The difficulties in technic most liable to be met by the practitioner are pointed out, and methods suggested as to how these may be overcome.

Stock analytic outfits selected by supply houses should not be purchased. The urinary analysis hand case will rarely, if ever, be taken from the physician's office, and is not adapted for practical work. Such selection should be made as will meet the requirements of the individual physician, reagents should be obtained in fresh condition, and both time and money will be saved.

Appropriate substitutes for the unavailable articles of equipment will constantly suggest themselves to the resourceful man, and the physician who, in an emergency, finds that he may inoculate a tube by means of a hat pin instead of a platinum point brings as much honor to the disciples of Esculapius as does he who ampu-

tates with butcher knife and meat saw the gangrenous limb of the frontiersman. As in other branches of medicine, **intellect first—equipment later.**

Substitutes for Gas and Running Water.

Gas.—The authors have demonstrated that gas is not only unnecessary, but sometimes undesirable, in laboratory work. In the large laboratory it is convenient, and, when satisfactory, should be used. The country physician has recourse to alcohol and gasoline. The small spirit lamp is inexpensive, may be taken to the bedside, uses only a small quantity of alcohol, and does not place him at the mercy of some gas plant engineer. There will be no "water in the pipes," no "cut outs," and no "meters." Various modifications of the alcohol lamp are marketed.

A portable burner with a wall tank can be had (Fig. 1), the reservoir holding about a quart of denatured alcohol, from which a flame four times as intense as that of the ordinary Bunsen burner may be obtained.

For continuous heating, a single-burner gasoline stove is ideal. A kerosene burner fails as a substitute, as its flame is usually inaccessible. A urotropin tablet, burned in the air, will heat to boiling the contents of three test tubes successively applied. This tablet should be placed on a glass or metallic plate, or in a medicine spoon. Poor grades of this drug have, however, a tendency to explode; throwing burning particles in all directions.

Gas is not necessary for incubators (page 21), and neither is it required for illuminating purposes. By properly manipulating the mirror, condenser, and diaphragm, smears and sections may be

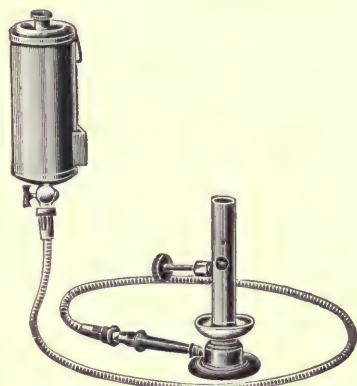


Fig. 1.—Giant alcohol burner. Its flame is four times as intense as that of the ordinary Bunsen gas burner.



Fig. 2.—Kerosene mantle burner.

examined by the ordinary kerosene lamp. The authors have tested with the most gratifying results the various mantle kerosene burners, one of which is shown in Fig. 2, and do not hesitate to recommend them to all microscopists as the best, and yet the most economical, of lights.

Running Water.—A city water supply may be aptly termed a laboratory luxury, and, when obtainable, is not to be despised. A siphon system, operated from a large bottle on a shelf, with a rubber tube and a pinch cock, will, however, answer very well. In staining, wash glasses (whisky glasses containing water) will save many steps. A large bowl of clean water serves well for washing sections and smears, as anilin stains, when much diluted, are practically inert. Blood pipettes may be cleaned without the aid of running water (see page 62). Centrifugalization need not depend on water pressure.

Essentials of Practical Microscopic Technic.

Mirror.—The plane mirror is the one usually employed, especially when examining stained preparations. The concave mirror is preferred when using artificial light or when examining tissue sections. In the latter case it is best to swing aside the condenser and narrow the aperture of the iris diaphragm.

Illumination.—Direct sunlight should never strike the mirror. The best angle for the reflection of light is as it comes from a white cloud (Novy). A white window shade will often be a great aid, especially in a south room.

Condenser.—Fig. 3 illustrates the portable microscope. The stage has been tilted in such a manner that the condenser and its control *B* are seen to best advantage. The use of the condenser usually comes with experience, but it should always be adjusted when beginning work. The man who “plays” with his condenser will soon become expert in its use. A correct focus of the condenser is as important as the objective focus. Neither increases the magnification, but both serve to render distinct the smear or section.

Iris Diaphragm.—This is rarely, if ever, opened wide. Correction of illumination is very often necessary in seeming indications for a widened aperture. With a slightly constricted diaphragm a stained object is rendered more distinct, and, when examining un-

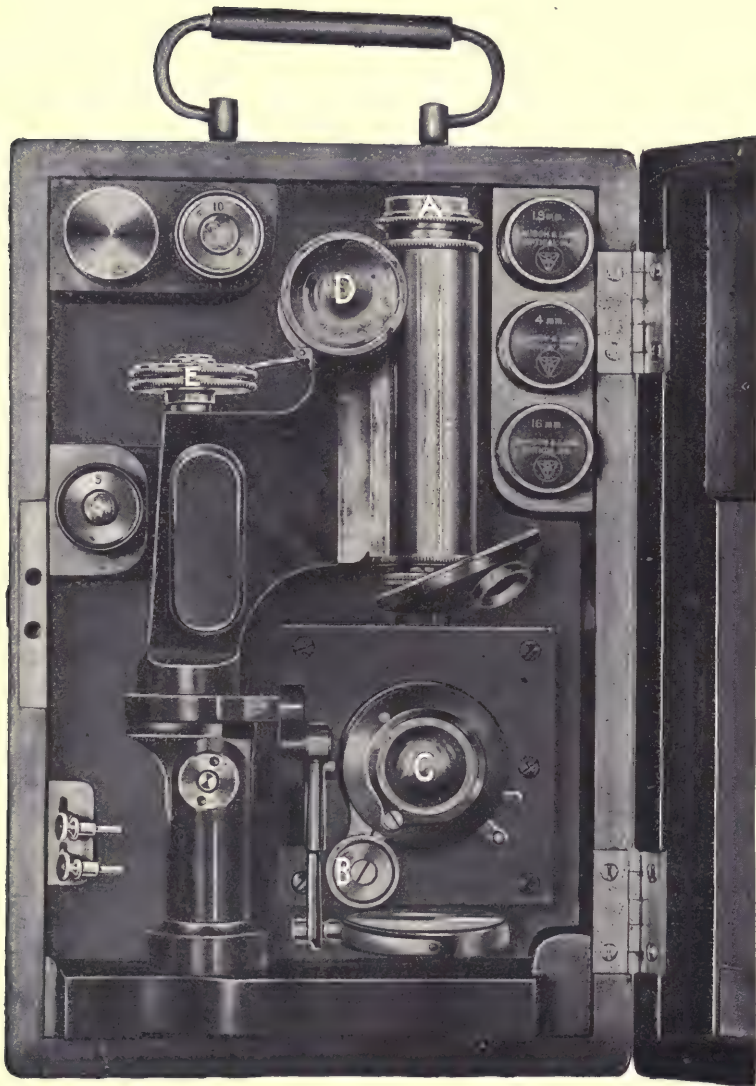


Fig. 3.—Portable microscope. A, to adjust draw tube; B, to control condenser; C, diaphragm; D, coarse adjustment; E, fine adjustment.

stained preparations, further narrowing is necessary, as that field is bathed in a dim twilight, the best possible illumination for such examinations.

Ocular.—This regulates, to some extent, the magnification. The

various oculars are numbered differently according to the make of the instrument. It is of advantage in diagnostic work to change oculars frequently.

Objective.—The use of the objective is usually so well understood that its description is omitted. The objective perfects the image, the latter lying just below the ocular.

Focusing.—This does not mean merely a lowering or raising of objective, but also an adjustment of the condenser. It is a good plan to make the final adjustment of the condenser after the objective is in proper position in order to obtain the most perfect image. It is usually advantageous to begin the study of a preparation with the lowest power and then proceed to higher magnifications. A good procedure for "safe" focusing is offered by Stitt: "It should be an invariable rule for the worker to bring his objective practically into contact with the upper surface of the cover glass, using the coarse adjustment to slowly elevate it into focus, and then maintain this focus with the micrometer screw."

Cleaning Lenses.—Expensive lens paper is unnecessary. An unstarched, but clean, linen handkerchief is much better. Oils are best removed with the aid of a similar cloth previously moistened in a little alcohol; if, however, it is applied to the tube, the alcohol quickly destroys the lacquer. Warm water and a soft cloth remove agar-agar and gelatin. Balsam is not so easily conquered, and must be avoided. Carbol-xylol will dissolve it, but its frequent use dissolves the cement which secures the lenses and may eventually loosen them.

Cover Glasses.—These should be used invariably, especially when working with the higher powers, as they render the object much more distinct. Prior to their use a drop of water or balsam must be placed on the preparation. Thick cover glasses interfere with high-power focusing and must not be used. Round cover glasses are used for smears of pus; for tissue section squares are preferable.

Microscopic Hysteria (Microscopic Cephalalgia).—This is not usually an idiosyncrasy, but results from one or more well-known causes. It may be due to an uncorrected error of refraction, to improper illumination, malposition of condenser, or an open diaphragm. A severe headache may arise from an accommodation squint. If one eye is closed, it soon becomes fatigued because the working eye must accommodate. A dark glass worn over the unemployed eye will often relieve this condition. **Both eyes should**

be open. It is a good plan to alternate the use of one eye with the other.

Laboratory Tables.—These should be painted black, but walls should be of a light tint.

Miscellaneous Rules.—The following rules should be carefully observed.

1. Use microscope in a vertical position, and slide clamps will not be necessary.
2. Use a chamois skin to polish lacquer.
3. Keep fingers off lacquer, and carry the microscope by its handle.
4. Do not lay a slide on the stage until its under surface is known to be dry.
5. A microscope, when not in use, must be kept from dust by placing it either in its case or under a bell jar.

A Bacteriological Laboratory for Five Dollars.

Five dollars or less will buy everything necessary for a physician's bacteriological laboratory—microscope not included. The bugbear in considering such a laboratory has been the incubator, and this matter has usually been presented to the general practitioner in a discouraging manner. The fact is, however, that all that is necessary is some method of maintaining cultures at a constant temperature of 98.6° F.—i. e., at blood heat.

During the hot summer months no such contrivance is necessary, and for other times of the year many substitutes for the \$50 gas incubator may be made. For example, a chicken incubator will serve the same purpose, a kerosene "germ warmer" that will answer is on the market, and a high candle power electric bulb may be immersed in a pan of water and the heat regulated by adding or removing water. A certain poultryman was able to hatch chicks in a bee hive from the heat given off by these insects, and, although the establishment of an apiary in a clinical room is not recommended, the method is not without a lesson. Perfect cultures of the diphtheria bacillus have been obtained in an office slightly overheated by an ordinary round stove, and an instance is known where a man carried a living culture of the typhoid bacillus safely through a blizzard by means of a special pocket in his underwear. A considerable fall in temperature, though inhib-

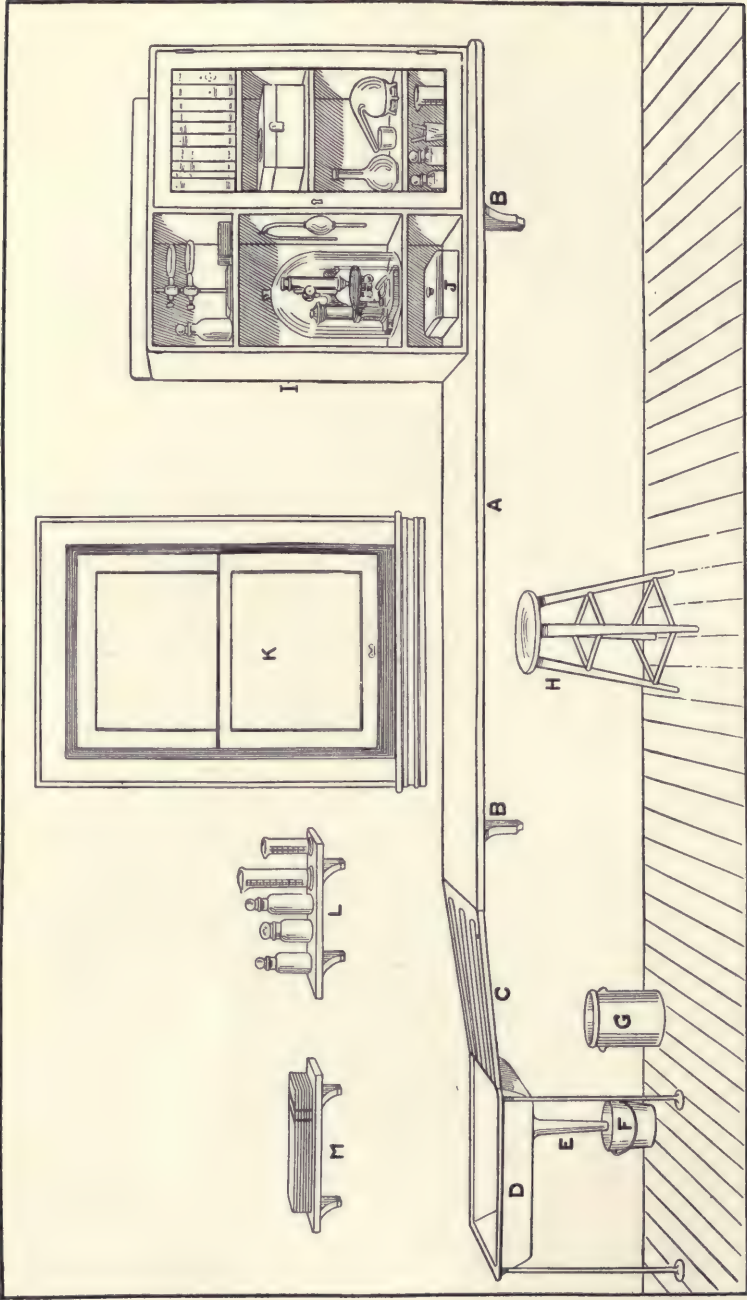


Fig. 4.—The laboratory side of a physician's consultation room. A, shelf table; B, brackets to support shelf table; C, drain board; D, sink; E, drain pipe; F, waste bucket; G, waste jar; H, stool; I, apparatus cabinet, one-half covered with glass for protection from dust; J, receptacle, with lid, for offensive specimens; K, window for microscopy; L, shelf for stains, etc.; M, shelf for towels.

iting the growth of many microörganisms, does not necessarily result in their death. These facts account for the possibility of mailing diphtheritic material to far distant laboratories, the retention of the virulence of "spoiled" ice cream, etc.

A fireless cooker will serve to keep plate cultures at a growing temperature, and for tubes the vacuum bottle has been recommended, which is not only convenient, but has the advantage of being well adapted for refrigerative purposes.

The hot air sterilizer may be easily dispensed with, as there is no better sterilizer for test tubes, metal ware, flasks, and cover glasses than a small gasoline oven, which may be heated with a gasoline or alcohol burner.

Culture media should be obtained from a reputable firm in assorted lots or a dozen, which will save considerable expense and trouble that would be incurred in an attempt to prepare them. After using these media the cultures may be killed and the tubes cleaned for chemical analyses.

The following estimate of prices of accessories should be considered by the practitioner:

Vacuum bottle	\$1.95
Gasoline oven	1.25
Alcohol stove	1.75
Six tubes of culture media.....	.30
Platinum loop	.30
	\$5.55

Arrangement for a Physician's Laboratory.

The idea that the clinical laboratory must be a separate institution is erroneous. Its location need not be seclusive, nor need it be even separated from the consultation room. One side of the ordinary examining room may be devoted to laboratory purposes, and the light side should, of course, be chosen. An ideal arrangement is shown in Fig. 4.

A shelf table, without legs, should be made to run the entire length of the laboratory space. Cypress is the best wood for its construction, and it should measure about 20 inches in width. It should be at a height convenient for the "standing" analysis, and a high stool, when desirable, may be used for the microscopic examinations.

At one end of this shelf table should be located the apparatus cabinet, and the sink is built into the other end. All apparatus should be kept free from dust in the cabinet, at least a portion of which should be covered with glass.

The shelf table should be securely fastened to the wall in order that there be no legs to trip the foot or thwart the broom, and a small quantity of black paint will add the finishing touches. Such a laboratory table will require little or no room, as examining chairs, bandage or dressing tables, etc., may be placed under it when these are not in use.

In case sunlight is direct and blinding, white shades may be drawn over the windows.

The shelf can be easily kept clean with a chamois, slightly dampened, and formalin added to the water not only inhibits or kills pathogenic microorganisms, but will destroy odors of pus, urine, etc.

Offensive specimens of excretions or bits of diseased tissue should be kept from the view of patients.

During a urinalysis the samples should be kept in amber or blue-colored glass bottles or jars. A "stinking" sample of urine is rarely excusable. Except in some forms of cystitis or gynecologic conditions, a freshly voided urine never smells. If, however, an analysis of a bad-smelling specimen is necessary, it should be deferred until an hour when visitors are most unlikely to enter. After such analysis there should be a thorough aeration of the room, for which purpose the following formula is a most excellent deodorizer:

R Iodoform.....5 j
 Oil of spearmint.....ad saturated solution
 Sig.: Nebulize, vaporize, or otherwise distribute throughout the room.

The physician should remember that most reagents are freezable, with the following notable exceptions: carbol-xylol; alcohol and alcoholic stains and solutions; ether; solutions containing glycerin—for example, Haines' solution.

Laboratory reference books should be kept with the laboratory equipment, ready for convenient reference. This book was not written for the bookcase, but was intended to lie on the table

within easy reach of the physician. On the laboratory walls may be hung charts of bacteria, solubility tables, etc.

Most solutions and stains should be kept only in small quantities on account of evaporation, precipitation, etc. Haines' solution should be prepared fresh in small amounts every few months.

Further laboratory suggestions have been made in succeeding chapters. For example, directions for the care of blood pipettes occur in Vascular Dramas (page 62), principles of asepsis and antisepsis occur in Searching for Germs (page 42), etc.

LABORATORY EXPERTS.

Before giving a description of the various tests it seems advisable to sound a word of warning in regard to a subject about which there seems to be much confusion—laboratory experts. Modern medicine, especially its laboratory branches, is built on a scientific foundation; in fact, its progress is a history of the progress of prophylaxis and diagnostics, each physician having, to some extent, his own ideas regarding therapeutics. There was a time when a certain mysticism was associated with sending a specimen to some prominent chemist for a test, but now medical students are trained to perform and understand these tests. A physician should perform all the tests which present knowledge and equipment place within his reach; but what shall he do when he is not prepared to make some important investigation?

Practitioners are cautioned against some laboratories. To state a diagnosis to a patient means to pass judgment, and is a serious matter. In case the physician has not the time or is not prepared for a certain investigation, he should exercise care as to whom he calls into consultation. In the first place, he should studiously avoid the ordinary chemist or the chemical supply house, and should seek the medical specialist. The institution which asks no remuneration for its services should be avoided, although this may not be advisable when dealing with patients unable to pay the necessary fees. A curt "positive" or "negative" from some student, or from a favorite of unknown ability, not only means nothing to the conscientious therapist, but such report is usually so misleading as to be dangerous. Indeed, if as much care were taken in the proper selection of consultants in diagnostics as in the field of surgery, or in the departments of ophthalmology and

otology, our medical successes would more than compensate for such efforts.

It may be well before leaving this subject to caution physicians against the sending of specimens to sanitariums or other institutions whose ultimate aim may be to secure for themselves the patients from whom the specimens were taken. The same caution will apply to reports from tent colonies concerning sputum examinations or to reports from certain mineral springs in regard to urinalyses, as these reports are liable to be biased and their recommendations be accordingly unsafe.

The physician should personally do as much as possible of his analytical work, and should spare neither time, money, nor study to become a safe diagnostician. The practical information presented in this book will aid him in his endeavors, and, in case expert help becomes necessary, the best help obtainable should be secured. Patients will appreciate intelligent treatment and will be willing to pay accordingly.

CHAPTER II.

THE SPUTUM.

Apparatus.—Does not include that used in the albumin test.

- | | |
|--|---------------------------------|
| 1. Glass plate. | 7. Carbol-gentian violet. |
| 2. Teasing needles (sharp hat pins
make excellent substitutes). | 8. Dilute nitric acid. |
| 3. Platinum loop. | 9. Very dilute acid fuchsin. |
| 4. Black laboratory table or card
board. | 10. Ordinary blotting papers. |
| 5. Slides. | 11. Several whisky glasses. |
| 6. Round cover glasses. | 12. Cover glass forceps. |
| | 13. Flame. |
| | 14. Microscope and accessories. |

Scope of Work.—The arrangement of apparatus is shown in Fig. 5. Those tests will be described which are most commonly needed by the practitioner, and less frequently applied procedures, including those usually left to experts, are merely listed.

Obtaining the Sputum.—Give the patient 15 grains of potassium iodid at bedtime, and, if possible, repeat this dose at about 2 o'clock the following morning. This drug is preferably given in milk. On rising in the morning the patient should wash out his mouth with a little soda water, being careful not to hawk up at the time any sputum. The sputum is then collected in a wide-mouth vaseline bottle. Saliva is not wanted. Sputum should be coughed up or raised by hawking. It is manifestly a waste of time to examine oral, nasal, and pharyngeal secretions for evidences of pulmonary involvement.

Quantity.—This is so variable in the several stages of lung diseases as to be of little diagnostic import. A very small quantity of true sputum is the rule in incipient tuberculosis.

Consistence.—Here, again, are variations. When the sputum is very thin and watery, showing a tendency to froth, there is a probability of pulmonary edema. Pus is easily divided into drops with the aid of a pipette, but mucus has a tendency to cling, not

References.—Sahli: Diagnostic Methods; Von Jaksch: Diagnostics; Boston: Clinical Diagnosis; Wood: Chemical and Microscopical Diagnosis.



Fig. 5.—Apparatus for sputum analysis.

only to other matter (adhesion), but is separated into drops with difficulty (cohesion).

Color.

Color.	Cause.	Significance.
Translucent and slimy..	Mucus	Variable.
Black	Soot	Normal in some occupa- tions.
Rusty, prune juice, or yellow	Changed blood	Usually pathological.
Green	Hemorrhage or icterus..	Pathological.
Gray	Pus	Pathological.
Red	Blood	Pathological.

Small clumps of a bright-green color may often be observed in influenza.

Odor.—Sputum is odorless, except when it becomes contaminated with putrefying microorganisms, which may be observed in pulmonary gangrene or bronchiectasis. Sometimes a sputum, after standing for a few hours, unless kept on ice, becomes soured. The authors have seen a drop of secretion from a fetid rhinitis contaminate an odorless sputum to such an extent as to render examination almost impossible.

Albumin Sputum Test.—Soluble albumin in the sputum is identified in much the same manner as in urine, but because of the presence of mucus, specimens of sputum are filtered with difficulty, so that it is necessary to modify the technic somewhat. In a large test tube, five drams of physiologic salt solution, five c.c. of sputum and five drops of acetic acid, are well shaken for five minutes. It is easy to commit to memory this technic when the quantities are thus remembered in fives, though the measurements do not need to be accurate. Shake well, filter and test the filtrate for albumin by any of the tests which may be applied for serum albumin in the urine. In chronic cases where uncompensated valvular lesions and nephritis may be ruled out, the positive reaction invariably means tuberculous involvement of the lungs as albumin is not met in chronic bronchitis. It is usually present in acute diseases of the lungs and bronchi, and is of practically no differential value except in protracted conditions.

Searching for Elastic Tissue.—Pour all the sputum on the glass plate and set this plate on a black surface for examination. A

laboratory table painted black will answer the purpose. Next tease out the solid portions of the specimen with needles. Bits of elastic tissue—i. e., lung tissue—appear usually as yellowish granules. Very rarely they may be tinged with red blood or may be hardened (calcification). These bits of tissue should be selected

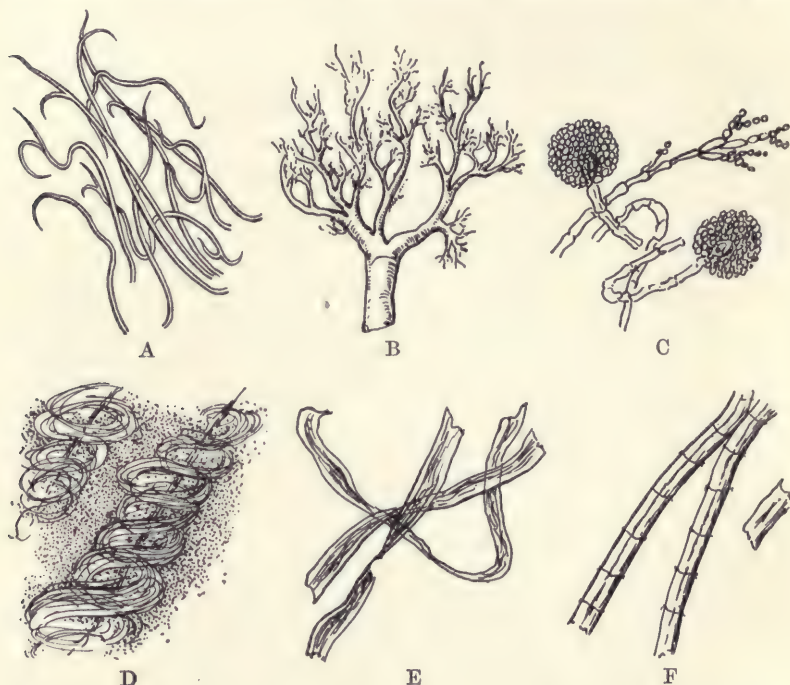


Fig. 6.—Elastic tissue and other sputum findings with which it may be confused. A, elastic fibers, with acute branchings and ends resembling fish-hooks, seen only when diaphragm is narrowed, and in a moderate light they show a double contour; B, fibrinous bronchial cast; C, molds; D, mucous spiral; E, cotton fibers, extraneous, and usually come from the towel used in wiping laboratory glassware; F, linen fibers.

out, and examined for the typical yellow fibers. Low power should be used, and, if the field is well illuminated, the diaphragm should be somewhat narrowed. Food particles, actinomyotic granules, tonsil plugs, etc., may be mistaken for these particles of lung tissue. Under low power the elastic fibers refract light in such a manner as to make them appear double, which can not be properly shown in a drawing. In Fig. 6 elastic fibers are shown, and their appearance is so characteristic that confusion with the other elements in this figure is rarely excusable. Their broken ends tend

to curl into a form not unlike that of a fish-hook, and branching at acute angles may also be noted. When once recognized, they do not usually cause confusion in future examinations.

Significance of Elastic Tissue.—Its presence in sputum means, invariably, the coughing up of diseased lung tissue, and signifies usually the presence of Koch's bacillus. Elastic tissue may sometimes be found in nontuberculous abscess, rarely in pulmonary gangrene, and **never in normal sputum.**

Preparing the Spreads.—Pick out suspicious particles with a platinum loop which has been previously sterilized in the flame and then cooled. Spread these well on clean round cover glasses and allow them to dry. Do not neglect to heat the loop to redness before laying it aside. When thoroughly dried, pass these cover glasses, with the specimen side up, rapidly through the flame several times, taking care not to burn the preparation. A brownish coloration indicates that the preparation has been ruined. The cover glass should, however, feel hot to the finger, and this heat fixation not only glues the preparation to the glass, but causes the stain to hold.

Staining the Preparation.—The following modification of the Ziehl-Neelsen method and its congeners has given the authors the best results:¹

Seize the cover slip with forceps and lock. Hold specimen side up, and cover it with a few drops of carbol-gentian violet. Hold above the flame in such a manner that it steams, but does not boil. This height is soon learned, and varies with the intensity of the flame. Replace with a medicine dropper any of the stain lost by evaporation. After steaming for three minutes, the excess stain is poured off and the preparation is ready to go through the washes. The latter are contained, most advantageously, in small tumblers, and the actual process is that of paddling gently each different liquid with the preparation. Each specimen should go through the following washes:

1. Water, one minute.
2. Dilute nitric acid until only a bluish tint remains.
3. Ethyl alcohol, 50-percent, two seconds.
4. Water **immediately** (not that used in 1), two minutes.

¹This same technic is applicable where the physician prefers to stain with carbol-fuchsin and counterstain with methylene blue or bismarck brown. It is occasionally advisable to apply both methods to the same specimen in case any doubt exists.

5. Acid fuchsin solution, one-half minute.

6. Water, two minutes.

Dry the preparation between two ordinary blotters and examine, mounted either in water or balsam. The tubercle bacilli should appear as small, slender violet rods on a pink background. Oil immersion objective should be used in the examination.

To Prepare Dilute Nitric Acid.—Add four drops of concentrated nitric acid to each half ounce of water.

Carbol-Gentian Violet.—Use the liquid stain as prepared by some responsible company.

To Prepare Acid Fuchsin Solution.—Use 1 part of the alcoholic solution to 20 parts of water. Filter if necessary. Dilute picric acid makes even a more beautiful counterstain, but does not stain properly other microorganisms. It should be used only by one who has learned to recognize the tubercle bacillus by its morphology as quickly as by its staining characteristics and who has thoroughly mastered the technic.

The Findings.—It is not sufficient that Koch's bacillus is absent or present. Is there a secondary infection, or is any purulent infection which may be present the primary condition? **What germ is causing the symptoms?** What is its degree of virulence? What stage of tuberculosis is present? Is there caseation or lipidification? Types of white blood cells are of some import, and many polymorphonuclears are more apt to indicate secondary infection than a few lymphocytes. Records of over one thousand analyses made by the authors indicate that correct diagnostic and prognostic conclusions were often obtained from corpuscular elements alone. The following table illustrates some of these findings:

Phthisis.		Purulent infections.
Elastic tissue	None.	
Lipidification (lung stones)	None.	
Bacillus tuberculosis	Nonacid fast cocci or bacilli, among which certain types usually predomi- nate.	
Caseous masses	None.	
Blood (red cells)	None.	
Lymphocytes	Pus cells—i. e., disintegrating poly- morphonuclear leukocytes.	

Appearance of the Tubercle Bacilli.—These tend to occur in clumps. Each measures in length about one-half the diameter of

a red blood cell, and often presents a beaded appearance or shows a slight curving of the ends. While branched forms have been observed, they rarely branch. In very virulent strains they may appear to be shortened even as coccus-like bodies (exaltation), and, when but slightly virulent, show tendencies to produce involution forms, as Schrön's capsules (attenuation).

The behavior of the tubercle bacillus to the acid, may be explained by the presence of mykol in the bacterial cell. Mykol is an alcohol and is doubtless combined with some fatty acid. Other acid-proof bodies exist and may contaminate a sputum. Among these are lycopodium dust, cork cells and honey-comb wax. These should cause no confusion if the physician searches for typical rods.

Less Frequently Applied Procedures.—These include those examinations rarely attempted and also those usually left to the expert. In rare instances the physician may desire to search for the actinomyces clubs or the echinococcus hooklets. A description of these should hardly occupy a place in this work, but the following list of less frequently applied procedures is given:

1. Curschmann's spirals in bronchial asthma.
2. Heart failure cells in valvular lesions.
3. Other vegetable parasites, as diplococcus pneumonia, bacillus influenzae, and certain molds.
4. Animal parasites, as ameba coli, etc.
5. Certain crystals of little or no diagnostic importance.
6. Albumin tests.

General Sputum Difficulties.—For those who have difficulty in finding Koch's bacillus, there seems but one remedy—be sure of the technic. The correct procedure may be determined by taking a sputum known to contain tubercle bacilli and making several dozen good stains from it. While making these stains the technic will be mastered. It is surprising how expert a physician may become after one afternoon's practice and how many hours of needless labor he may avoid in the future.

Some persons do not work with a system, and a negative result will be discouraging simply because a repetition of the work means another half hour of application to the technic. Apparatus should be arranged to save time, and should be kept clean and properly classified for use. Several spreads may be made and fixed at one time, so that if the first stain fails it will not be necessary to hunt up the sample—or possibly the patient—for another test.

Difficulties in Spreading.—There is a tendency to spread too thickly. A droplet of the sputum—not a drop—should be used. An attempt should be made to cover every bit of the cover glass surface, and a film so thin as to be almost invisible is most likely to give the best results.

Difficulties in Drying.—These difficulties are more serious than may appear at first thought, and it is in this part of the technic that many failures occur. It is obvious that a thin spread dries much quicker than a thick one. Drying should, however, be thorough, and it is not sufficient that no water be visible. There may be some moisture present—enough to interfere with proper fixation. It is a safe rule to wait at least twenty minutes after all moisture has apparently disappeared before fixing the specimen.

Difficulties in Fixation.—These difficulties have been emphasized under description of the method of fixation (page 31). Incineration, or overfixation, is recognized by the loss of characteristic morphology of the spread elements. Underfixation is not impossible, and is shown by a tendency of the smear not to stain, or, when stained, to readily lose the dye. When, after staining, any motions of the spread elements are observed, the physician may feel certain that the fixation has not been complete. Although the proper fixing temperature is learned by experience, a safe rule is that the cover glass be hot, but that the preparation be not burned.

Difficulties in Staining and Washing.—If the stain contains sediment, filter it, and, if it seems to be too thin, obtain some fresh stain. Although it must steam, ebullition renders the specimen unfit for examination because pieces of it are dislodged and float off in the stain. Do not allow any portion of the preparation to become dry, but add a drop of stain as needed. Two or three minutes usually suffice for this procedure. Pour off the excess stain and wash **immediately** in water. Let this washing be thorough, so that any precipitated stain may be removed, as such particles may be easily mistaken for caseated masses, or even microorganisms.

When the nitric acid causes the stain to fade appreciably, dip twice in dilute alcohol and **immediately** wash briskly in clean water. It may be added that one of the most beautiful stains ever seen by the authors was made by a physician who, finding that his supply of alcohol had become exhausted, substituted gin.

If a light-blue remains, transfer to fuchsin, but, if the prepara-

tion is still dark, wash once more in the nitric acid solution. The preparation may not be entirely ruined even if no blue is discernible, and it should not be decolorized again merely because a few blue lumps occur. These overstained areas indicate thick spreadings, and should not be taken into account at the final examination.

Difficulties in Mounting.—The slide should be clean. A droplet—not a drop—of balsam should be used. Apply the cover glass lightly, and do not press it into place. In the colder months the balsam may not spread, and, if such be the case, warm the slide **slightly**. If an air bubble clings to the balsam droplet, a pin prick will cause its disappearance.

To Reclaim a Preparation.—Several smears should be spread and fixed at one time. If the preparation has been mounted in balsam, it may be difficult to put it into a suitable condition for restaining, and soaking it in carbol-xylol may aid in removing the balsam. One unaccustomed to this work may examine the stains in water instead of balsam.

Value and Limitation of the Sputum Analysis.—Tubercle bacilli are often absent in incipient phthisis, but the number of such instances recorded has been improperly increased by the reports of persons who used faulty technic or made wrong observation.

It is often advisable to substitute for the fuchsin counterstain an aqueous picric acid solution. This does not, however, properly stain the other microorganisms, but, if the technic has been thoroughly mastered, this disadvantage is more than offset by the ease with which the tubercle bacillus may be demonstrated—a bright-purple rod on a lemon-yellow background. A deeply stained epithelial cell to which Koch's bacillus shows a tendency to cling can not hide it if this method, as proposed by Mix, is followed.

The finding of elastic fibers is indicative of "coughed-up" lung, providing the vomiting of meat foods has not occurred during the coughing up of the sputum, a matter that should be determined.

It may be accepted as a rule that the finding of acid fast bacilli in the sputum indicates pulmonary tuberculosis. Other acid fast bacilli are more likely to be found in pharyngeal secretions than in true sputum. The smegma bacillus does not show a curling of the ends, nor does it lie in clumps. In case of doubt, other acid fast bacilli may be excluded by decolorizing with the alcoholic solution of hydrochloric acid described under tuberculosis of the urinary organs. (See *The Urine in Disease*, page 123.)

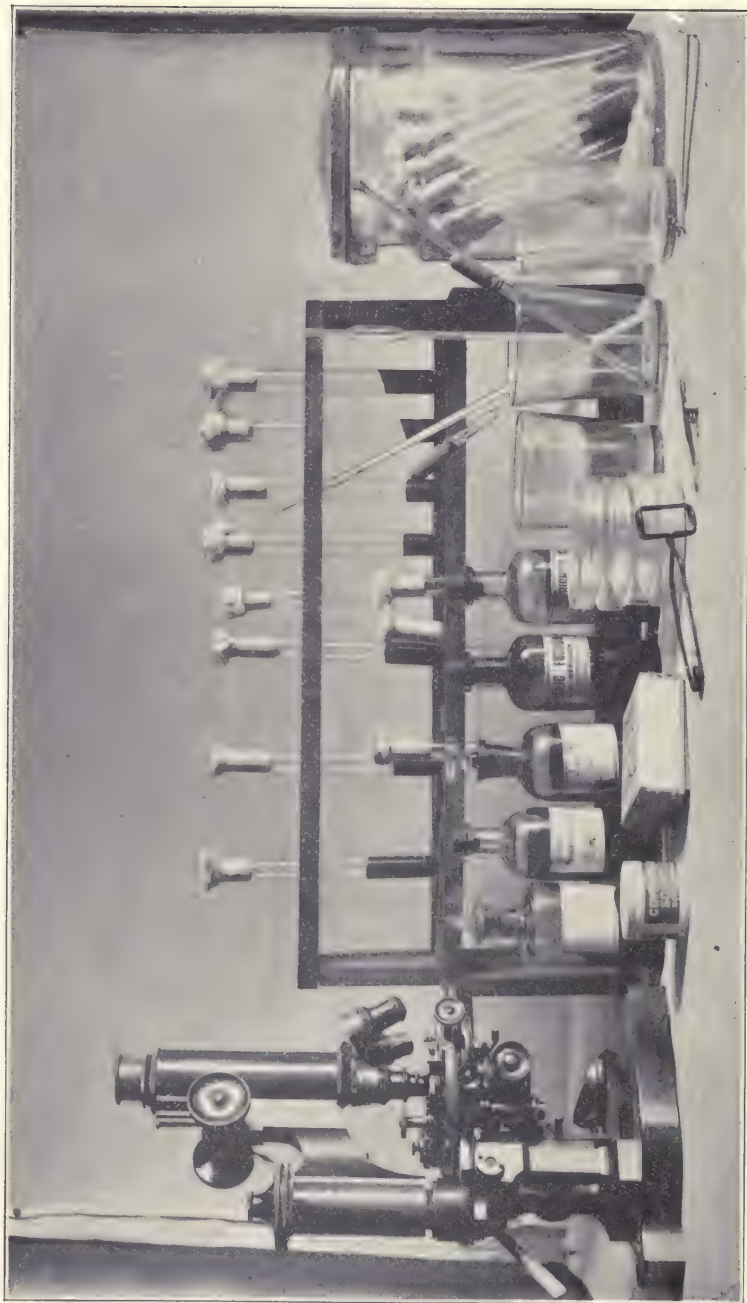


Fig. 7.—Apparatus for bacteriological examinations.

CHAPTER III.

SEARCHING FOR GERMS.

Apparatus.—

- | | |
|---|--|
| <ol style="list-style-type: none">1. Aqueous solution of 10-percent sodium hydroxid.2. Cover glass forceps.3. Culture media, prepared and sterilized. (See page 23.)4. Dish of 1:1,000 bichlorid of mercury solution, colored blue.5. Flame.6. Gasoline oven designed for one burner.7. Incubator or substitute. (See page 21.)8. Microscope and accessories.9. Mason jar for unused culture tubes. | <ol style="list-style-type: none">10. Ordinary Fahrenheit thermometer.11. Pan for boiling water.12. Petri dishes.13. Platinum point or loop, or both.14. Slides and cover glasses.15. Stains and pipettes—methylene blue, Löffler's methylene blue, Wright's blood stain.16. Glass tumbler with cotton in bottom for supporting test tubes, platinum wire, pipettes, etc.17. Uterine spoon curet. |
|---|--|

The arrangement of apparatus is shown in Fig. 7. In this chapter will be described in a plain, but thorough, manner some of the bacteriological examinations which may be made by the country physician. Investigations requiring much time, expense, and considerable skill are not included in these examinations, and animal inoculation experiments are avoided. The study of the micro-organisms of the bacillus tuberculosis, treponema pallidum, diplococcus intracellularis, and plasmodium malarie will be found in The Sputum (page 32), Vascular Dramas (page 67), Exudates in Brief (page 97), and To Find the Treponema in Six Minutes (page 173).

Culture Media.—These may be obtained from certain pharmaceutical houses in assorted tubes of a dozen and properly sterilized for use. The following selection should be kept on hand and available at a moment's notice:

References.—Jordan: General Bacteriology; Stitt: Practical Bacteriology; Novy: Laboratory Bacteriology; McFarland: Bacteriology; Muir and Ritchie: Bacteriology.

Nutrient agar, four tubes.

Löffler's serum agar and swabs, four tubes.

Glucose agar, two tubes.

Nutrient gelatin, two tubes.

These media should be kept in a glass jar free from dust, and the rubber caps which prevent the ingress of the air should not be removed until the media are to be used. After a few months these media "dry out" and should then be replaced with new material.

Influences Which Inhibit Germ Growth.—

1. *Absence or presence of oxygen*, depending on whether germ is aerobic or anaerobic. The only anaerobic germ considered in this book is the tetanus bacillus.

2. *High or low temperatures*. The body temperature is most favorable to the cultivation of pathogenic microorganisms.

3. *Light*. Bacteria work best in the dark, and sunlight is their worst enemy.

Inoculation of Tubes.—With a bit of the material on a sterile platinum wire, inoculation of suitable media may be accomplished by any one of several methods:

1. **HORIZONTAL STREAK.** The contaminated wire is drawn across a plate (Petri dish) of culture media. Each germ develops a colony where it happens to fall.

2. **STAB.** The contaminated wire is pushed down the center of solid culture media in a tube. Such a method is well adapted for anaerobic germs.

3. **INCLINED STREAK** (Fig. 8). This is a very convenient method for the rural worker, as purchased media in tubes are usually slanted. The wire bearing the suspected microorganisms is drawn along the center of this surface from the bottom upward. The following technic is followed when inoculating by this method:

First. Sterilize the wire in the flame and hold with the right hand in such a manner that contamination is improbable.

Second. The tube is held in the left hand



Fig. 8.—A streak culture.

between thumb and index finger, with plugged end toward the right. It should be held in a position almost horizontal, so that it may not be contaminated by falling dust particles when the plug is removed.

Third. Remove and discard the rubber cap. The cotton plug is grasped between the middle and third fingers of the right hand and quickly removed with a sharp twist.



Fig. 9.—Correct method of inoculating a tube.

Fourth. Hold the neck of the tube in the flame for a moment in order to incinerate any microorganisms which may be attempting to gain entrance.

Fifth. Now inoculate the tip of the sterile wire with the **smallest** bit of the suspected material and streak the agar as described above, being careful not to contaminate anything (Fig. 9). Especially avoid air currents, and do not allow the platinum point to touch the side of the tube.

Sixth. Withdraw wire.

Seventh. Again pass neck of tube through flame.

Eighth. Insert cotton plug.

Ninth. Sterilize wire in flame.

The entire technic, when properly learned, should take only about one minute, and should be conducted with care. The inoculated tube should be kept at body temperature for twenty-four hours.

Small colonies should mark the path of the wire. Unless the least amount of the suspected material has been used, these will, because of their great number, coalesce and render isolation of the causative germ impossible. A platinum point is, therefore, more desirable than a loop. Separation, if necessary, must be done at once, as overcrowding as well as the presence of involution forms will occur as time passes.

Isolation of Pure Cultures.—Specimens from the various colonies may be examined at once, and this is usually an advisable procedure in diagnostic work. It may, however, be the desire of the worker to obtain a pure culture of the microörganism—i. e., where the tube contains only this germ and coalescence of colonies does not cause contamination—which may be done as follows:

First. Place several tubes of nutrient agar upright in a can of boiling water. When liquefaction of the media has occurred, permit the water to cool down to about 122° F. This is the inoculating temperature, solidification occurring as cooling progresses below this point.

Second. Meanwhile, with a hand lens, study the colonies on the original slanted surface. Some may be round and others irregular, some transparent and others opaque, and some flat and others raised. A certain germ invariably gives rise, in its growth, to a colony typical of its species, and inoculations from a single colony should result in a pure culture of that germ. In case all colonies appear alike, the primary inoculation was possibly a pure culture.

Third. When the melted agar reaches 122° F., inoculations should be made at once according to the method described above. In this case a platinum point, rather than a loop, should be dipped into the colony, as otherwise too many microörganisms will be obtained. Each inoculation should be mixed well into the agar, and the wire resterilized in the flame before inoculating the next tube.

Fourth. The liquid agar, thus inoculated, is immediately (after heating neck of tube) poured into a Petri dish that has been previously heat sterilized. The tube is then thrown into a strong

solution of mercuric chlorid and later cleansed by boiling. The Petri dish is immediately, with its contents (plate preparation), set aside at blood heat. Other dishes are inoculated in the same way.

The above technic requires haste, as the agar cools rapidly and solidifies.

Authors' Short Method.—The authors are convinced that, for practical purposes, the above plating is not always necessary. The process is tedious, and, though usually described as the standard method, offers so many opportunities for contamination that the practitioner is justified in seeking further for a substitute.

In this book the statement has been emphasized that, although isolation of pure cultures is not a necessary procedure in many diagnostic problems, the worker soon takes pride in this separation. If, however, such isolation is avoided, an early examination of specimens from the colonies is often imperative for the reason that coalescence of colonies and involution forms of the germs occur. A simple method of isolating pure cultures is to select samples from the various colonies and inoculate other agar slants. When this work is done early and carefully, it will not be subject to criticism.

Incubation in Vacuum Bottles.—Reference has been made to substitutes for the expensive and complex sterilizer and incubator. If a so-called vacuum bottle or a fireless cooker be employed, it will be necessary to see that the cultures are obtaining a proper amount of oxygen. Such an incubator should be kept in a warm room, and the cork or lid inserted only after both the culture and the inclosed air are warm. The required warmth may be secured with a slightly heated stove. It may be advisable to have this temperature a little higher than 98.6° F., as some cooling will always occur. In order to insure plenty of oxygen, it is best to "reincubate" at least once during the twelve hours or twice during the twenty-four hours. A little sweet oil heated to body temperature may be placed in the bottom of the vacuum bottle, but plenty of air must be left above the surface for the use of the developing colonies.

Sterilization.—1. NUTRIENT MEDIA. These should be purchased ready for use.

2. GLASSWARE AND METALWARE. These should be boiled for at least ten minutes, making sure that all portions are touched by the

water, after which they are cleaned and dried. Contamination with various atmospheric bacteria occurs during the cleaning, so that Petri dishes and other glassware treated in this manner are not available for further culture work without **hot air sterilization just before using**. The sterilization may be conducted in a gasoline oven, provided that sufficient time and care be taken. The degree of heat required—about 300° F.—necessitates removal of all cotton plugs and organic material. Glassware, but not metalware, may be immersed in a strong solution of mercuric chlorid before boiling, which does not usually kill the germs, but weakens them to such an extent that they easily succumb to the boiling water.

3. COVER GLASSES. These, when cleaned and dried, may be passed through the flame several times preliminary to making a spread, and may be heated past the fixing point, so that all organic material is destroyed. After cooling, they are practically sterile and the spread may be made at once.

4. TOWELS. These may be dipped into the mercury solution, but a thorough boiling should never be omitted. During the boiling every portion should be immersed in the water.

5. HANDS. During culture work the hands should not come in direct contact with any sterile apparatus, nor with any suspected virulent material. The fingers should not touch the platinum loop, lower end of the cotton plug, inside of the tubes, or Petri dishes known to be sterile, and should not, of course, come in contact with colonies, pus, etc. At frequent intervals the hands should be scoured with a brush and hot suds. Unless mercuric chlorid causes dermatitis in the worker, he may keep a 1:1,000 solution conveniently near, into which to dip his hands occasionally, and thus inhibit the effect of any germ which might alight at the mouth of a sweat gland. For convenience of distinction, a little blue or green coloring matter may be added to this solution. The physician who looks on these solutions not as antiseptics, but as disinfectants, has something to learn.

6. METAL INSTRUMENTS. Except for the mercury solution, which ruins them, these may be treated in the same manner as glass. Forceps, wires, etc., may be heated to incandescence in the nonluminous flame.

7. LABORATORY TABLES. Should be wiped off at frequent intervals with a cloth saturated in 1:500 solution of mercury.

Simple Stains.—Of these the authors prefer methylene blue in most diagnostic procedures, as this dye usually stains well, but seldom overstains. Other good stains are fuchsin and gentian violet. The methylene blue stain is prepared by diluting some of the concentrated alcoholic solution with water, the object being to obtain a stain with little alcohol. It is best, however, to dilute slowly until the liquid is transparent, as otherwise precipitation of the stain will occur on the cover glass, and, if this happens, reject the stain and make up some more, using less water. An unsaturated stock solution gives, when diluted, a weak stain, and hence the necessity of obtaining these dyes from a reliable firm. The following technic for staining bacteriological smears will give excellent results:

First. Spread thoroughly some of the material on a sterile cover glass with a sterile platinum loop. This wire is always sterilized by heating to incandescence in the nonluminous flame. Remember that a spread is rarely or never too thin, but is often too thick, or at least may be so in spots.

Second. Dry in air.

Third. Fix in flame according to directions given under sputum (page 30).

Fourth. Permit preparation to cool.

Fifth. Grasp the glass with forceps and lock, and, holding specimen side up, add enough stain to cover the smear.

Sixth. After about one minute—exact time depending on strength of stain—wash off the dye under the tap or dip repeatedly in a bowl of water.

Seventh. Dry lower surface of glass with a piece of filter paper or an ordinary blotter, invert, and float, specimen side downward, onto a clean slide.

Eighth. This serves as a diagnostic examination. In case it is desired to keep the preparation, it may be dried between two blotters and mounted in a droplet of balsam (page 34).

Staining Errors.—Overstaining with methylene blue is rare. Understaining, however, is common, and may depend on one of four causes—overfixing, underfixing, a weak staining fluid, or insufficient time of exposure. Overfixation is usually apparent by the injury to the bacteria—charring, etc. Underfixation permits the floating off of the specimen during washing. Underfixation may be microscopically demonstrated by the motility of the germs, even

though partially stained. In case staining troubles should continue, it is possible that the solution is too dilute, and precipitation of the dye may indicate this condition. In case the staining time was insufficient, the preparation should take the dye on the second trial.

Other Staining Methods.—There are many excellent stains indicated in other books, but which can hardly be recommended in this work as absolutely necessary for the diagnostician, who must reserve many of his energies for other kinds of labor. In searches for the gonococcus and diphtheria bacillus, the physician may advantageously substitute Löffler's methylene blue. Gram's double stain, spore stains, flagella and capsule stains—and indeed many others not mentioned in this chapter—should be understood by the practitioner in order that the value and limitations of all bacteriological work may be borne in mind.

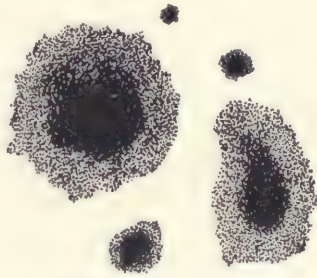


Fig. 10.—Surface colonies of diphtheria bacillus. These become quite characteristic within eighteen hours, while the other microorganisms may not yet be visible. The colony is flat, with a wavy edge and a dark center. The contents are grayish white, coarsely granular, and not unlike ground glass. Drawn from an eighteen-hour culture.

Searching for the Bacillus Diphtheriæ.—At least one physician in the radius of every one hundred miles should be prepared for this examination, and no county seat should be without a person equipped for this work. The procedure is not difficult, and, if the examination is conducted by the physician in charge of the case, it will be more valuable than if made by a person—often not a physician—several hundred miles distant.

A swab from the throat may be tested immediately, as smears can be made, stained, and examined at once. To be certain of the diagnosis, an inoculation may be made which, within eighteen hours, should show hundreds of the Klebs-Löffler bacilli. Isolation in pure cultures of this microorganism is not necessary for the

diagnosis of diphtheria. The serum agar slant tubes and swabs may be purchased with other media. No disinfecting solutions are to be used on the throat preliminary to the swabbing, for which the procedure is as follows:

1. Rub swab over affected portion of the throat, removing, if possible, some of the membrane.

2. Streak over surface of serum agar. Several such tubes should be so prepared.

3. Keep at blood temperature from eighteen to twenty-four hours.

Diphtheria Colony.—This is moist in appearance, large, round, and of grayish color (Fig. 10). The center is thick, while the edges are thin, and examination with a hand lens shows a wavy border. Cover glass smears may be made from these colonies, fixed, and stained with Löffler's methylene blue.



Fig. 11.—Some of the more common forms of the diphtheria bacillus, reproduced from actual preparations.

Bacillus Diphtheriæ.—The Klebs-Löffler bacillus shows some or all of the following characteristics as compared with other bacilli:

1. Irregularity of form and size, illustrated in Fig. 11.
2. Club shapes, or bacilli bearing swollen ends.
3. Cross striations or bands.
4. Monopolar or bipolar staining.
5. Wedge shapes.
6. Curved forms.

Certain forms of bacilli—pseudo types—may closely resemble the true bacillus of diphtheria. In case of doubt their character can be determined by allowing them to come in contact with a drop of water, when the pseudo type will form a cloudy suspension, which will not occur with the true Klebs-Löffler bacillus.

Searching for the Bacillus Tetani.—The cultivation of the tetanus bacillus requires the absence of oxygen. Many methods

have been devised, but the method given here is suggested, and smears from the wound may be examined. It is a well-known fact that anaerobic germs may multiply in the presence of those requiring oxygen, a phenomenon that is termed "microbic association," and therefore, if any tetanus bacilli in the wound are capable of multiplying—unless planted very deeply in the tissues—the presence of certain oxygen-requiring germs is presumed.

1. With a sterile uterine spoon curet scrape well the wound, being certain that no antiseptic has been used previous to this procedure. A thorough cauterization for therapeutic purposes should follow this curettage.

2. Add some of this material to a tube of serum agar—not a slant, but one which has been previously liquefied, if necessary, and resolidified in a vertical position (page 40).

3. Incubate from four to seven days.

4. Examine for characteristic drum-sticks, which are not easily found, for, even if the culture be pure, all tetanus bacilli do not show the end spore, and typical drum-sticks often escape detection because simple stains do not bring out well the end spore. Hot carbol-fuchsin will stain both the spore and the body. (For method see page 31.) The tetanus bacillus, with or without a spore, is usually a very long and slender rod.



Fig. 12.—Typical fir tree tetanus stab in gelatin. Six days' culture. Occurs only in the lowest portions of the inoculation.

5. Heat the culture in water up to 175° F. and maintain at this temperature for forty-five minutes. Only spores remain viable.

6. After cooling, make a stab culture in glucose agar and incubate. Such tetanus spores as may be present will develop deep in the agar and show a cloudy growth, with characteristic perpendicular branchings. Its indistinctness, together with the fir tree appearance, is peculiar to the microorganism. The gelatin stab culture is much more distinct (Fig. 12).

Sources of Error.—The isolation of the tetanus bacillus is not an encouraging procedure, even for the expert. Strange to note, however, the authors have seen it identified by persons who would be least expected to find it. The attempt is worth the effort,

although the spores of other anaerobes may interfere. Stitt attaches importance to the odors which may arise from the serum culture, and states: "From day to day smell the culture, and, if an odor similar to the penetrating sour, foul smell of the stools of a man who has been on a debauch is detected, it is suspicious. The nondevelopment of a foul odor is against tetanus."

Searching for Koch-Weeks Bacillus.—This may be necessary when attempting to differentiate a severe catarrhal conjunctivitis from a mild gonorrheal infection. Smears from the secretion should be made and stained for the gonococcus (page 48). If the Koch-Weeks microorganisms, instead of the diplococci, are present, they will appear as very short bacilli. If the gonococci are present, they usually, though not invariably, lie within the protoplasm of the pus cells.

Searching for Streptococcus Pyogenes.—This important micrococcus has been found in the following conditions:

1. Diffuse inflammations, as phlegmons, erysipelas, etc.
2. Inflammations of respiratory tract, as pharyngitis, bronchitis, lobular pneumonia, etc.
3. Acute articular rheumatism.
4. Ulcerative endocarditis.
5. Puerperal fever.
6. Scarlet fever.
7. Certain inflammations of serous linings, as peritonitis, etc.

Some authorities claim that the streptococcus pyogenes is a distinct microorganism, being closely related to other chain cocci, but not identical with those causing erysipelas, rheumatic fever, and scarlet fever. So far as diagnostic purposes are concerned, this distinction is unimportant.

The streptococci occur normally in the mouth, nasal cavity, large intestine, vagina, and on the integument. The attenuation and exaltation of the streptococcus seems an easy matter, and the resisting powers of the organism need suffer only slight decrease to render it an easy prey. In pus, search first for the staphylococcus and then for the streptococcus, but follow vice versa in the diffuse and more extended inflammations. The isolation and identification of this coccus by cultural examination is hardly to be recommended to the country physician, as there are so many sources of error and so many limitations. Smears stained with methylene blue offer a much better, though still imperfect, method. Usually,

though not invariably, the long chains of streptococci, compared with the short chains of less than eight cocci, are the really dangerous forms, but it has been proven that virulent short chains do exist.

Searching for Staphylococci.—This is the most common of all pus germs, and for practical purposes need not be divided into subclasses. It is, however, interesting to note that furuncles and other abscesses of the integument are due usually to the varieties which form yellow colonies, while those of pharyngeal infections are white. Suspected pus may be inoculated into nutrient agar by following the directions applying to diphtheria. The opaque creamy-white or light-yellow colonies are most likely to show the staphylococcus. Both forms liquefy gelatin, and often the germ may be found in the pus smears.

While methylene blue serves well as a stain, the use of Wright's blood stain, when much work is being done with pus, is specially recommended as an occasional substitute for methylene blue, for, if one stain is misplaced or spilled, the worker will not be forced to discontinue the examination. Wright's stain, though requiring distilled water, does not make heat fixation necessary.

Isolation of the Typhoid Bacillus.—With our present knowledge the practitioner should be content to leave this work to specialists. At best it is tedious, and, when attempted from suspected water, is usually a very discouraging procedure.

Pneumonia Versus Tuberculosis.—"Galloping consumption" may resemble lobar pneumonia, and the differentiation of these conditions is considered under sputum analysis. The pneumococcus is very small and is seen only with the highest powers. The capsules may best be demonstrated in hanging drop preparations. Cultures are unsatisfactory.

Searching for the Gonococcus.—No cultures should be attempted. Löffler's methylene blue stains well, as does also Wright's blood stain. The typical form of this coccus, its occurrence in pairs, and its tendency to lie within the pus cells are its characteristics, and it is not necessary to use Gram's stain in order to avoid confusion with other micrococci.

Oil immersion objective should be used. Three drams of gin, taken twelve hours before the examination, usually drives this germ out of its hiding places in the gland ducts.

Searching for the Colon Bacillus.—A germ which, once con-

sidered harmless, now has the distinction of chief etiological factor in the following conditions:

1. Some cases of cystitis.
2. Some cases of peritonitis, especially those resulting from perforation of the bowel or appendix.
3. Some cases of appendicitis.
4. Some cases of mucous colitis.

The bacillus coli communis is a normal and, doubtless, a necessary inhabitant of the large bowel. Neither the morphological nor the cultural properties of this microorganism are sufficiently characteristic to justify its search. The presence of rods in urine does not, as is commonly supposed, warrant the diagnosis of colon cystitis. For its relation to sewage contamination see *Some Simple Water Analyses*, page 145.

Searching for Molds.—There are three very common pathogenic molds:

1. *Microsporon furfur* of fawn chest, or tinea versicolor. Scrapings from the skin should show, besides epidermal cells, the hyphæ of this mold, and intermingled are loose spores which show a tendency to clump formation.
2. *Endomyces albicans* of thrush; segmented hyphæ, with spore formation within; and branchings may be seen at segments.
3. *Trichophyton* of barber's itch, ringworm, etc.; a number of varieties showing hyphæ.

Therapeutic indications make an absolute classification of these varieties for the practitioner unnecessary, and it is sufficient to find hyphæ or spores, or both. The following technic has given excellent results:

1. Make scrapings from the edge of the lesion, and never from the central portion.
2. Drop these on a clean slide, and add 2 or 3 drops of 10-percent sodium or potassium hydrate solution.
3. Apply lightly a clean round cover glass and let stand fifteen minutes.
4. Firmly press down the cover glass, flattening the specimen.
5. Examine with a somewhat contracted diaphragm and a high-power objective.

Searching for Actinomyces.—Besides the ordinary "jaw infections," cases of consumption, ischiorectal abscess, and furunculosis have been observed in which the chief etiological agent seemed to

be the ray fungus. Cultivation for diagnostic purposes is hardly worth while. The characteristic yellowish granules may often be observed in the pus without the aid of a lens. Smears may demonstrate the characteristic clubs; if they do not, curettage of the walls of the abscess may loosen the fungus.

Other Searches Which May be Attempted by Smears Alone.—

Culture work is unnecessary in many bacteriological examinations. Many of these have been included in other chapters—viz., treponema, tubercle bacillus, plasmodium, and meningococcus. Others may be attempted by the smear method, as:

1. Spirocheta of Vincent's angina; in certain throat ulcers.
2. Ameba coli and cholera vibrio. (See Every-Day Stool Tests, page 150.)
3. Bacillus influenzae from greenish expectoration.
4. Bacillus pyocyaneus from green pus.

Researches.—All medical truths have not originated in medical schools or hospitals, and there is no reason why a general practitioner should not have discovered the treponema pallidum just as a country doctor first identified the germ of tuberculosis. As an illustration of what is yet to be discovered, the following list of diseases—of an infectious character, but of doubtful or unknown etiology—are submitted to the country physician for his consideration, of which diseases the leaders of scientific research have thus far failed to find the cause, although much expense has been incurred by investigations:

1. Infantile paralysis.
2. Pellagra.
3. Rabies.
4. Acute articular rheumatism.
5. Measles.
6. Mumps.
7. Scarlet fever.
8. Smallpox.
9. Whooping-cough.
10. So-called colds.

Difficulties and How to Avoid Them.—Many sources of error are self-evident, and need no consideration, while some errors are made in following the various methods. If, however, the technic of culture work is closely followed, there should be very little trouble.

Value and Limitation of These Searches.—Both value and limitation are variable, not only as to the various microorganisms, but also as to the physician—that is, a procedure which may be of value in the hands of one person will be a waste of time in the hands of another. A physician who has not finished a course in laboratory bacteriology should be slow to handle the more virulent germs, and, on the other hand, the late graduate may attempt searches not recommended in this book. A safe rule will be, **before reaching any conclusion as to the identity of any microorganism or its relation to the pathological process at hand, to thoroughly study the subject from the best works on bacteriology**, as in this book only those examinations have been selected and classified which may be profitably employed by the general practitioner.

Searches Left to Experts.—In the light of our present knowledge of this relatively new subject, it appears that the practitioner may wisely avoid working with some germs, as follows:

1. Germs identified with difficulty: colon bacillus, typhoid bacillus, paratyphoid bacillus, germs of food poisonings, dysentery bacillus, tetanus bacillus.
2. Germs exceedingly virulent: plague bacillus.
3. Germs rarely found: anthrax bacillus.

Laboratory Prophylaxis.—For additional information on this subject see Laboratory Prophylaxis, page 179.



Fig. 13.—Apparatus for blood examinations.

CHAPTER IV.

VASCULAR DRAMAS.

Apparatus.—

- | | |
|---|---|
| 1. Acetic acid, glacial. | 13. Slides and cover glasses. |
| 2. Acetic acid, 1-percent. | 14. Slide forceps. |
| 3. Alcohol. | 15. Sodium chlorid, aqueous solution,
.85-percent. |
| 4. Broom straw. | 16. Sticker or stub pen. |
| 5. Distilled water. | 17. Stiff paper. |
| 6. Ether. | 18. Tallqvist hemoglobin chart. |
| 7. Glycerin. | 19. Towel. |
| 8. Hayem's solution. | 20. Wright's blood stain; in 1-ounce
bottles in liquid form from
some reliable chemical or op-
tical firm. |
| 9. Horse hairs. | |
| 10. Marx's fluid. | |
| 11. Microscope and accessories. | |
| 12. Red and white pipettes and count-
ing chamber. | |

The arrangement of apparatus is shown in Fig. 13. With a little practice any physician may become expert in the various blood analyses. While some examinations are of importance, they must not be overestimated, as a diagnostic chain usually requires many strong links before it becomes useful to the therapist, and only the few most valuable procedures are recommended. One method, the best and probably the most simple, is described briefly, but no necessary details are omitted, and sources of error are pointed out unless self-evident. The actual selection of the necessary examinations for a given case lies with the physician, it being hardly worth while to make complete blood examinations in every case. In this selection the diagnostician must not go astray, and it is unwise, for example, to conclude that, inasmuch as a low hemoglobin is observed, the red count would be diminished and the counting of the erythrocytes neglected.

Development of the Blood Cells.—It is probable that all blood cells, red or white, normal or pathological, originate from one

References.—Ewing: Pathology of the Blood; Schleip: Atlas of Hematology; Cabot: The Blood; Da Costa: Clinical Hematology; Watkins: Diagnosis by the Blood; all works on clinical diagnosis, including Sahli, Simon, Boston, Wood, Emerson, Webster, etc.

parent cell, and, with the exception of many of the lymphocytes, all blood corpuscles are formed in the red bone marrow. The spleen, once termed the "cradle and the grave" of the blood, by no means fills either office, but may serve often as a brooder for the younger corpuscles and as a subheart—a "portal pump,"

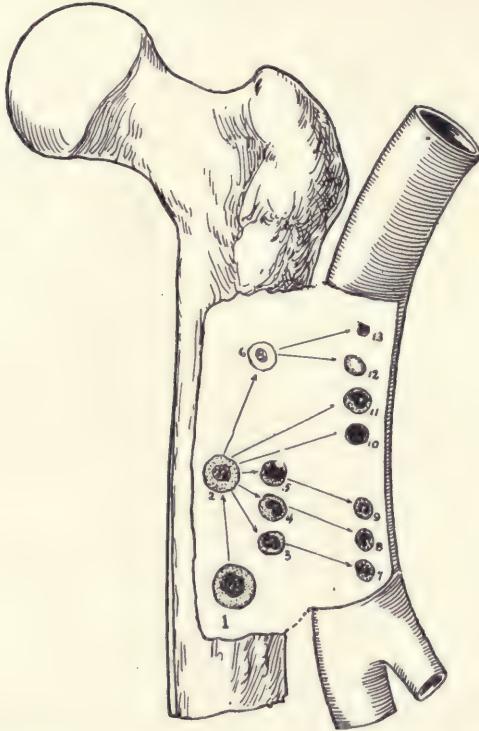


Fig. 14.—Development of the blood cells. Schematic representation of the corpuscles normally found in the red bone marrow, showing how they give rise to the various circulatory elements. Cells normally present in bone marrow: 1, parent cell; 2, intermediate forms, with neutrophilic, basophilic, and eosinophilic alterations; 3, neutrophilic myelocyte; 4, basophilic myelocyte; 5, eosinophilic myelocyte; 6, erythroblast. Cells normally present in circulation: 7, neutrophilic leukocyte; 8, basophilic leukocyte; 9, eosinophilic leukocyte; 10, lymphocyte; 11, transitional; 12, erythrocyte; 13, blood plate.

which, by the slow contractions of its muscular walls, forces the blood through the liver to the central pumping station. Hepatic rather than splenic tissue forms the tomb for the erythrocytes.

A modified form of certain diagrams offered by Schleip, with nomenclature according to the suggestion of Grawitz, is given in Fig. 14.

Blood Dramas.—For convenience of description, we may consider each blood picture a “drama,” the circulatory system the “stage,” and the red bone marrow the “back of the scenes.” In the normal blood picture the various circulating corpuscles are of constant variety and in practically unvarying number, and in differential counts there are no marked percentage alterations. In disease, however, the scene is changed and there is turmoil at once. In these conflicts certain types may be deformed or destroyed, others may be increased in number, and finally, in the so-called crises, those cells not ordinarily observed in the circulating fluid may be called from the home or the brooder to aid their struggling offspring. It must, therefore, be concluded that the so-called blood diseases do not give rise to corpuscles hitherto not found in the circulating blood or in the blood-forming organs. Every morbid blood scene has, for its characters, dead, dying, or wounded normal cells along with certain other varieties in increased numbers—active or injured maternal reinforcements. By keeping these points in mind, the significance of each blood smear can be realized without difficulty, and the pathology of blood diseases will be easily comprehended by the clinician.

Behind the Scenes.—Here is the busy spot. For example, in pernicious anemia, as the red cells are destroyed, there is call for reinforcements. The organism demands even more oxygen than usual, and an extra strain is put on the bone marrow. More and more red cells are rushed into the circulation, but to no avail. Finally the bone marrow becomes fatigued—if we may use such term—as those storehouses in the spleen, hemolymph glands, and perhaps other organs and tissues have been exhausted, and the demand exceeds the supply. The result is an inferior product, or, to adhere more closely to our comparison, “understudies.” Cells, hardly fitted for the assumption of the duties thrust upon them, are rushed to the fray. Among these are found the oversized and undersized cells, forms showing mitoses and red cells which have not yet cast aside their nuclei. They are quickly destroyed, and their dying forms—polychromophilia, poikiloblasts, etc.—must not be mistaken for cells entirely foreign to the organism. Then the bone marrow rouses and concentrates its energies in one mighty effort; there results the blood crisis, the sending out of certain forms not only unfitted by improper development for the struggle, but often already diseased—the megaloblasts. In

this manner a little study may make plain the picture or scenes of any blood disease.

Cast.—The complete description of blood corpuscles will be found in the larger books, but a few brief hints may not be amiss. In all cases where the colors of stained preparations are considered it is to be understood that reference is made to Wright's stain. Although blood cells appear flat when spread, fixed, and stained, such is not their appearance in the natural state. All white cells are globular, the polymorphonuclear forms showing pseudopods. The red cell is not a biconcave disk, as was the former conception, but is thimble-shaped—i. e., one surface is very convex and the other deeply concave, such morphology being a direct result of the extrusion of the nucleus of the erythroblast. These nuclei, in turn, are supposed to give rise to the blood platelets.

For convenience, the blood corpuscles have been arranged in systematic order, and only those characteristics observed in the stained preparation are considered:

Red Cells.—

1. Erythrocyte; the normal red cell; orange or pink.
2. Undersized cell; sometimes normal.
3. Microcyte; very small erythrocyte; invariably pathological.
4. Oversized cell; sometimes normal; in large numbers are characteristic of pernicious anemia.
5. Macrocyte or megalocyte; very large red cell; this and remaining forms of red cells are always pathological.
6. Poikilocyte; irregular form, with beak or snout.
7. Shadow or ghost; loss of hemoglobin, with distended cell wall.
8. Endoglobular degenerations; appear as unstained droplets within the protoplasm.
9. Enlarged delta.
10. Small delta.
11. Normoblast; with single deep-blue nucleus.
12. Microblast; small normoblast.
13. Macroblast or megaloblast; macrocyte, with pale nucleus and signs of degeneration.
14. Poikiloblasts; nucleated poikilocytes.
15. Degenerating normoblasts; showing lobulated nuclei.
16. Forms showing karyorrhexis; granular disintegration of nuclei.
17. Forms showing karyokinesis; division figures in nuclei.

18. Polychromatophilia or paradoxical stain; where nuclear dust of karyorrhesis mixes with hemoglobin, causing a bluish tint.

White Cells.—Percentage refers to the differential count in adult's blood.

1. Lymphocyte (19 percent); robin's-egg-blue cytoplasm; round purple nucleus.

2. Transitional (3 percent); light-blue cytoplasm; horseshoe nucleus.

3. Polymorphonuclear eosinophile (2 percent); cytoplasm contains large and deep-red granules; lobulated nucleus.

4. Polymorphonuclear basophile (1 percent); cytoplasm contains medium-sized, light-blue granules; lobulated nucleus.

5. Polymorphonuclear neutrophile (75 percent); cytoplasm contains small reddish-lilac granules; lobulated nucleus.

6. Myelocytes containing the various colored granules; these are very large cells, with single nuclei; pathological.

7. Degenerating leukocytes; cytoplasm contains vacuoles; pathological.

8. Disintegrating leukocytes; disappearance of cytoplasm, with a scattering of the granules; pathological.

9. Necrotic changes; nuclei stain poorly or not at all, or else show fragmentation.

Blood Dramas.—The table given on page 58 serves to differentiate the more common blood conditions. For detailed information reference should be made to the larger books.

Bedside Apparatus. In case the patient is bedfast, suitable specimens of the blood may be obtained and taken to the office for examination. Dilutions and spreads may be completed with little apparatus, which may be easily carried in the physician's hand-bag, as:

1. Towel.
2. Blood sticker or stub pen.
3. Hemoglobin chart.
4. Red and white pipettes.
5. Catheter tubing.
6. Vial containing Hayem's solution
for reds.

7. Vial containing (1-percent) acetic
acid for whites.
8. Vial containing alcohol.
9. Six clean slides and some stiff
wrapping paper.

The towel should be clean, and a clean towel or handkerchief can usually be obtained at the home of the patient. A nickel-plated German blood sticker offers no advantage over a stub pen

with one point broken off. Arneill recommends small rubber catheters instead of the tubing supplied with blood pipettes, and, if these are sufficiently long, the free end may be brought around and slipped over the point, in which condition the diluted and prepared blood may be transferred to the office and examined at leisure. A blood spread which has dried may be wrapped in heavy paper and taken to the office.

THE MORE COMMON BLOOD CONDITIONS.¹

Disease or condition	Red cells	Hemoglobin	White cells	Cytology
Chlorosis	N	—	N	
Polycythemia	+	N or +	N	
Secondary anemia	—	—	N or —	(a) Post-hemorrhagic—undersized red cells, few poikilocytes, some endoglobular degeneration, normoblasts. (b) Cachectic or toxic—microcytes, undersized cells, more poikilocytes, more degeneration; normal and degenerating normoblasts.
Pernicious or large-celled anemia	—	—	—	Loss of hemoglobin greater than above, moderate degeneration, oversized cells, macrocytes, megaloblasts, polychromatophilia, mitoses, degenerating normoblasts, less poikilocytosis than above.
Leukopenia	N or —	N or —	—	A condition in typhoid, acute miliary tuberculosis and pernicious anemia; white count decreased due mainly to diminution in the polymorphonuclears; lymphocytes are increased.
Leukocytosis	N or —	N or —	+	Noted in purulent infections; polymorphonuclears actually increased; lymphocytes relatively, but not actually, diminished.
Leukemia	—	—	+	(a) Lymphatic—increase of the lymphocytes. (b) Myelogenous—increase of the polymorphonuclears, appearance of myelocytes in the blood.
Eosinophilia	—	—	+	Increase of eosinophilic polymorphonuclears; noted in trichinosis, asthma, uncinariasis, menstruation, positive tuberculin, certain skin diseases, etc.
Hodgkin's disease	—	—	—	Picture of a cachectic secondary anemia.
Lymphosarcoma	—	—	+	Large cells or cells of various sizes, resembling very much the lymphocyte, but staining poorly or showing degenerations.
Iodophilia	N	N	+	Certain iodine reaction seen in the polymorphonuclears when purulent processes are present in the body.

Obtaining the Blood.—Although the lobe of the ear furnishes blood in considerable quantity, it may be often necessary in bed-fast patients to use the finger tip. A cold, bloodless integument does not represent that actual blood picture which may be present at other parts. If, however, a massage is undertaken, it is best to

¹ N, normal; +, increased; —, diminished.

wait until the deep-red color is replaced by the normal tint. The puncture should be made quickly, and should be fairly deep, so that repetition will not be necessary.

Macroscopical Examination.—Except in aggravated conditions, such an examination is of little use in diagnosis. A mushy blood may be due to a severe leukemia, a “whey” solution may suggest a great decrease in the number of cells, a pale blood signifies hemoglobinemia, and chocolate or crimson tints may be due to certain poisons. Without a suitable chart, colors are often misleading, and the laity watch with interest the changes in the color of the blood when in reality tests would show that there is no alteration in the hemoglobin.

Microscopical Examination of Fresh Blood.—Experts are often able to pass a final conclusion from a mere inspection of a hanging drop. Although the practitioner may make certain interesting observations, no final diagnostic inferences should be drawn from this procedure, and, if time is of importance, he may well omit this examination.

Hemoglobin Estimation.—For practical purposes, the Tallqvist color chart is sufficient. The original booklet form has a decided advantage over its modifications, as it is convenient to carry and is not easily soiled. Instructions accompany each booklet. The comparison of the color of the droplet with that of the chart should be made at once, as certain changes take place, on drying, in the hemoglobin. In case the physician has no hemoglobin chart with him, he will not be entirely at a loss, as comparison of a drop of the patient's blood with his own on a piece of white blotting paper may bring out good color distinctions.

Suspension of Red Cells.—Blood, after coagulating in pipettes, gives considerable trouble, and the beginner may find it advantageous to practice with a little carmine water. Dilutions must be made quickly. Wipe away any blood which may be present at the puncture, and, when the next drop appears, immerse well the point of the red pipette below its surface and draw, by mouth suction, a solid column of the blood to the mark 0.5. Quickly wipe off the point and instantly immerse it into the vial of Hayem's solution. Now quickly draw up this fluid until the mixture reaches 101. While this is being done the pipette should be twirled between the fingers, thereby insuring a thorough mixing of the liquid. Stop suction and remove rubber tube. With a thumb closing one

end and the middle finger closing the other, shake well. The dilution is complete, and the free end of the catheter may be pulled over the point, as suggested above. After reaching the office, another shaking is advisable, as the cells show a tendency to settle to the more dependent portions. Some persons prefer to remove the tubing entirely before leaving the home of the patient, and slip around the ends of the pipette a wide rubber band, which serves to prevent any leakage.

Errors and Difficulties.—Solid columns of liquid are imperative. Air segments occur when the drop is too small or when the point of the pipette has not been sufficiently immersed. It may be impossible to draw any fluid into the tube, which indicates a plugged pipette, due either to an old clot or a piece of dirt, which may be seen by the use of a hand lens or with the naked eye. A little glacial acetic acid and a horse hair may establish a lumen, and, in case these fail, a fine hypodermic needle may be used, but care must be exercised that the point of the pipette is not broken off. The same methods may be employed when a column of the blood has coagulated in the tube. In case the initial blood column passes the proper marking, a towel touched quickly, but skillfully, to the point may take up the excess of blood and bring the top of the column down to the proper mark.

Red Count.—After discarding four drops of the diluted blood, one small drop is blown into the clean counting chamber and the cover glass applied. The contact of the glass surfaces must be perfect, and this is shown by Newton's rings—a play of rainbow colors—seen best from an almost horizontal reflection of light. If the drop is too large, it spills out of the well and prevents the appearance of the rings. In such case the rings may be brought out by pressure on the cover glass, but will disappear if the pressure be removed. Permit the preparation to stand undisturbed for five minutes, so that the corpuscles may settle into the pens.

There is practically no difference in the various rulings so far as the inner counting pens are concerned. Butler proposes that when the lines become faint they may be rendered very distinct by scraping off some graphite from a soft lead pencil, rubbing the powder over the surface of the disk, and then polishing it off with a soft handkerchief.

The authors have tried with varying success at least half a dozen methods of counting red blood cells, but feel that still another

method must be offered to the person who, although desiring the best results, must, for the sake of his patient, proceed with more haste than is usually required of many laboratory workers. The technic of this other method consists of a combination of several methods, and possesses the following advantages:

1. Simplicity.

2. By avoiding the square field methods, it obtains more nearly an average of the entire dilution. The most careful workers too often complain that the count in one area often varies exceedingly with other counts, and consequently many fields must be counted in order to find—what is, after all, the main result desired—**an average.**

3. Prevents “losing the place.”

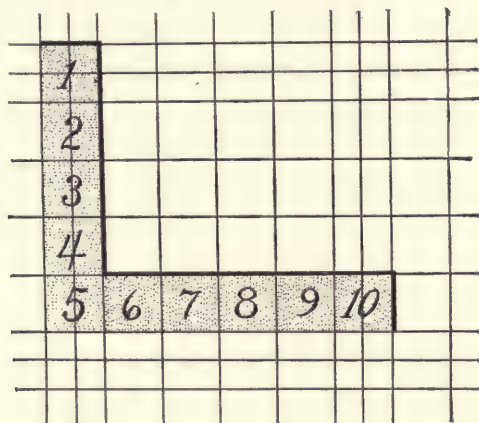


Fig. 15.—Scheme for rapid counting of red cells.

SIMPLE METHOD. Only four careful counts are made, and each of these includes ten squares, or forty in all. This may seem too few, but, if each of the four counts is made in a different portion of the preparation, there will be greater probability of striking an average than where several square areas are counted and the fact overlooked that, in spite of the best efforts, the cells lie more closely together in one area than in another.

All corpuscles on the heavy lines and within the shaded areas are counted (Fig. 15). The beginning position is self-evident, and may be any one of a number of points, the new Turk ruling giving a choice of one hundred and forty-four such counts. This method guards against “losing the place,” a complaint that is old and well

founded. By using the proper ocular and drawing high the tube of the microscope, the field may be greatly magnified. Always use a low-power objective and narrow the diaphragm. In normal blood each of the little squares should average about 6.2 cells, which number is increased in polycythemia and decreased in anemia. An estimate of the number of red cells in each cubic millimeter of capillary blood should be made, and this computation, for diagnostic purposes, need not be accurate, but, while watching the progress of a blood disease, considerable care must be taken. Add the four countings, multiply by 2, and add four ciphers to the product. For example, suppose the four counts were respectively 60, 59, 57, and 60. The sum of these would be 236; multiplying by 2 gives a product of 472; adding the ciphers, we have 4,720,000 cells for each cubic millimeter. This should be done in a very few minutes, and, it is safe to say, no better average of the entire ruled disk could be taken.

Cleaning Pipettes.—This should be done as soon as the count is finished, and the following method gives the best results:

1. Attach rubber tubing to the pointed end of the pipette, removing it from the other end.
2. Remove the remainder of the blood solution by blowing through the rubber tubing.
3. Immerse the large end of the pipette in dilute acetic acid, suck the liquid into the pipette, and blow it out, repeating the procedure with absolute alcohol.
4. Suck some ether into the pipette.
5. Remove rubber tube and hold pipette firmly in hand.
6. Because of the moisture ordinarily present in the exhaled air, ether can not be expelled by blowing, but by swift downward jerks of the hand.

Only in case the glass mixing ball shows no tendency to adhere to the inner surface of the pipette, can the latter be placed away as clean. It may be necessary, if blood is still present, to repeat the entire technic, but, if only moisture interferes, the acetic acid wash may be omitted. Stronger acids, horse hairs, or even fine needles may be employed to remove the more obstinate clots.

White Count.—Various bedside technics, where a hand lens is used, have been devised. They may serve some persons well, and should not be condemned, but the authors adhere to the use of the microscope for the following reasons:

1. The older methods are simple, and white counting does not usually require haste, except possibly in infectious cases.

2. More accurate results are obtained when privacy is secured and a microscope is used. Correct counts are not easily obtained in the environment of the sick-room.

3. The microscope always distinguishes between cells and dirt, and also between red cells and white cells.

It will, therefore, be seen that the rough methods of counting white cells are subject to serious limitations. The special white



Fig. 16.—Spreading. After the blood has run along the edge of the spreader, the drop is pushed over the surface of the other slide.

cell pipette and 1-percent acetic acid as a diluent are used, the technic being identical with that described for the red cells. The acetic acid destroys the hemoglobin, so that only the white cells may be seen, and brings out the nuclei of the white cells. Much yellow sediment indicates that the acid is not strong enough. It is often necessary to filter the acid solution at intervals of several months.

Draw up the microscope tube to such a distance that the periphery of its field cuts exactly the corners of the large square

millimeter, and permit the tube to remain in this position. In different portions of the preparation take five counts, including in each of these every white cell in the field. The sum of the five fields divided by 2, and with two ciphers added to this quotient gives the number of leukocytes in each cubic millimeter of blood.¹ For example, suppose that the counts are 29, 35, 35, 32, and 35, their sum would be 166; this divided by 2 gives 83; adding two ciphers gives 8,300. This rapid method was proposed by F. J. Wright, of the Calumet and Hecla Hospital, Michigan.

Making the Spread.—The most satisfactory method of preparing blood films is that in which the slides, rather than cover glasses,

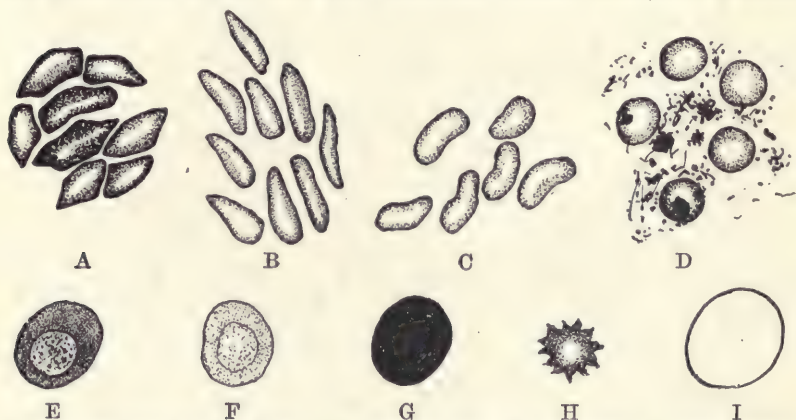


Fig. 17.—Artifacts in blood films. A, pressure forms; B, elongations; C, poor fixation; D, dirt; E, normal stain; F, understaining; G, overstaining; H, crenated cell; I, ghost or shadow.

are used. The following technic takes the preparation to the staining:

1. Touch the edge of an end of a clean slide to a blood drop and transfer it to the face of another slide near its end.
2. When the drop spreads along the edge of the smearer, push it over the face of the second slide (Fig. 16).
3. Dry in air.
4. Wrap in stiff paper and carry to the office for staining. If left exposed to flies, they will rapidly eat off the corpuscles.
5. No fixation is necessary when using Wright's stain.

Artifacts in Erythrocytes.—(Fig. 17.) Where fields are too

¹A dilution of 1:40 is necessary. This proposition has reference to the large form of the white blood counter, which has a capacity of 21. In case, however, the small form of the white pipette is used, which has a capacity of 11, corrections must be made accordingly, but the former pipette is recommended as being more convenient.

crowded, certain irregular pressure forms may suggest poikilocytosis. Parallel elongations of cells are due to pulling them out while making the smear. Bean-shaped forms are a direct result of poor fixation. A piece of dirt—usually black, rather than blue—may, by virtue of its position on an erythrocyte, suggest a normoblast. Endoglobular degeneration is made evident by the tendency of certain erythrocytes to take the stain rather poorly. If, however, all the red cells appear pale, there is sufficient reason to conclude that the preparation has been understained. In case over-staining takes place, the delta may not be easily recognized.

Staining the Blood Film.—The Wright's stain is simple, its use rapid, and its action all that can be desired. A little practice is, however, necessary, and the following technic will give the best results:

1. Cover the film with a noted quantity of the staining fluid dropped quickly from a pipette or medicine dropper.

2. After two minutes add to the staining fluid on the film the same quantity of distilled water with the medicine dropper, and allow the mixture to remain exactly two and one-half minutes. If the stain is all right, this should give an intense coloration to the cells. A longer period of staining invariably produces a precipitate. Eosinophilic granules are best brought out by a shorter period of staining. The quantity of the diluted liquid on the preparation should not be so large that some of it runs off. The water may show a tendency to gather in drops and roll off the stain, so that it may be necessary to mix it thoroughly with the latter before all is added. An ordinary medicine dropper full of the stain and the same amount of the water are usually sufficient.

3. Pour off the liquid and wash the preparation in ordinary water for thirty seconds, or at least until all precipitated stain is washed away and the preparation assumes a pink color.

4. Dry between blotters.

5. In several places, as may seem advisable, add cover glasses by means of balsam. If square or oblong cover slips are used, practically all of the preparation may be covered.

Authors' Slide Forceps.—Any one who has worked with the slide smear in preference to the cover glass preparation has been at a disadvantage in staining, as the Novy locking forceps, as well as the other varieties, can not be used with the ordinary slide. The authors have devised locking forceps which are sufficiently power-

ful to render the drop staining method applicable to slide spreads. In the illustration (Fig. 18) a regular hemostatic forceps has been used, and the bends not only permit point coaptation, but guard against crushing the slide.

Examination of Spreads.—This consists of a study of a stained smear under low and high power, especially under the latter. Proper diagnoses may be made by reference to the table on common blood conditions (page 58). For a description of the staining properties of the corpuscles see page 56.

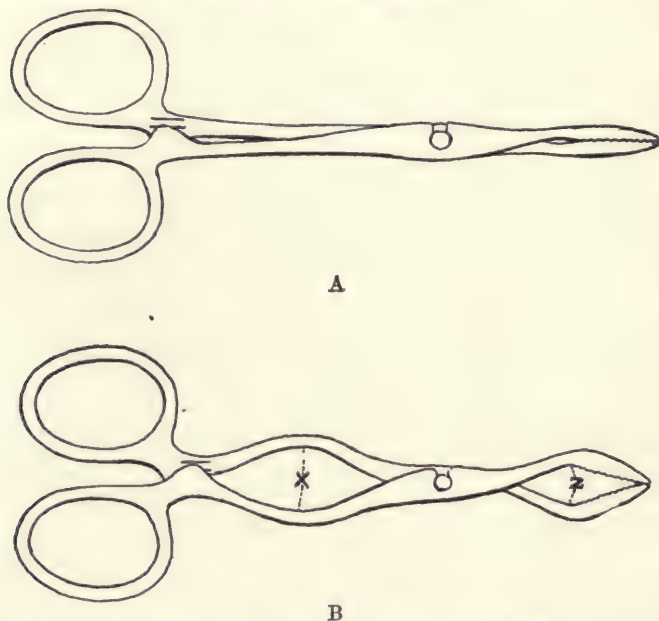


Fig. 18.—Authors' slide forceps. A, Pean's hemostatic forceps; B, shows alteration; X, prevents crushing of the slide when locking; Z, permits point coaptation.

Differential Leukocyte Count.—Certain diagnostic and prognostic data may be obtained from the determination of the percentage of the various types of white blood cells which may be present—an absolute increase in the polymorphonuclears being termed a leukocytosis, a decrease being termed a leukopenia, etc., each of the varied pictures representing some fact of clinical importance.

For this work a mechanical stage is an aid, but not a necessity,

the latter statement being a direct contradiction of that usually made in text books. At least 300 white cells should be classified unless the probable condition is self-evident. The computation is easily made. For example, if 255 of these 300 cells were lymphocytes, it follows that these constitute 85 percent of the entire white count—probably a lymphatic leukemia, etc. Whether a mechanical stage is or is not employed, the worker must never attempt to encroach upon a field whose cells have been previously numbered.

Parasites and Microorganisms.—The presence of the plasmodium is easily demonstrated by Wright's stain, which offers not only the most simple procedure, but brings out the most beautiful color contrasts. The body of the parasite stains blue, while the chromatin varies from lilac to red, or almost black. The chief source of error is mistaking blood platelets for endoglobular forms of the plasmodium, as the former often lie upon or even within the red cell, but are of a homogeneous blue color. The plasmodium, when really seen, is rarely mistaken for anything else. It is not the purpose of this book to enter into a discussion of the morphology of this microorganism.

To Prove Presence of Blood.—Take a stain.

1. Place some of the stain on a glass slide.
2. Add 1 drop of very dilute (.85-percent) salt solution.
3. Evaporate very slowly at a low temperature.
4. Add 4 drops of glacial acetic acid.
5. Apply a clean cover glass.
6. Again evaporate very slowly at a low temperature; fluid should steam, but not boil.
7. Add, twice, 3 drops of glacial acetic acid, and evaporate each time.
8. Cool.
9. Elevate cover glass and add 1 drop of glycerin.
10. Examine.

Blood, if present, is shown by the presence of dark-brown rhomboid crystals, which may vary in size, and there will be a tendency of these crystals to rosette formation. This is called the hemin test.

To Differentiate Fowl's from Mammal's Blood.—Take a blood clot. Tease out some of the clot in Marx's fluid and examine under the microscope. Marx's fluid is made up as follows:

R Quinin hydrochlorate, 1:1,000.....	2 drams.
Potassium hydrate solution, 33-percent.....	2 drams.
Eosin, yellow	about 5 drops.
Misce.	

Red cells should be stained pink. Those of the mammal appear as unnucleated circular disks, but those of birds and reptiles are oval and nucleated.

To Prove Human Blood.—This can be done only by the precipitin test, a difficult procedure, calling for expert assistance.

Determination of Coagulation Time.—This may be roughly computed as follows:

1. Allow several drops of fresh blood to fall on a clean glass slide.
2. At intervals of a minute make tests by drawing a smooth white broom straw lightly through each drop.
3. The coagulation time is reached when threads of fibrin tend to cling to the straw. The average coagulation time is about five minutes, and when it reaches or exceeds ten minutes it may be considered pathological, and operative procedures should be attempted with caution. In jaundice and hemophilia this time is greatly increased. The coagulation time may be experimentally or therapeutically shortened by the administration of gelatin or the calcium salts.

Widal Test.—This is described in *Diazo Versus Widal*, page 104.

Diagnosis of Carbon Monoxid Poisoning.—Treat the sample of blood with twice its volume of 1.3 specific gravity solution of caustic soda. Normal blood is changed to a dirty-brown, but, if carbon monoxid is present, a beautiful cherry-red will be seen.

Tests Seldom or Never Attempted by the Practitioner.—

1. Opsonic work.
2. Wassermann reaction and its modifications.
3. Detection of iodophilia.
4. Searches for tropical microorganisms.
5. Blood cultures; searching for microorganisms in the blood other than the plasmodium.
6. Diabetes tests.
7. Medico-legal work.

Value and Limitation of These Tests.—A correct interpretation of these tests, with a more or less complete application of the various procedures, will prove of inestimable value in many diagnos-

tic and prognostic difficulties. A "blood analysis" should not, however, be overestimated, as a blood picture may be ever so beautiful and characteristic, teeming with symptomatic therapeutic indications, and notwithstanding this appearance fail to indicate the chief etiological factor. It is often only by a series of examinations that the case in question may receive a rational, scientific treatment.

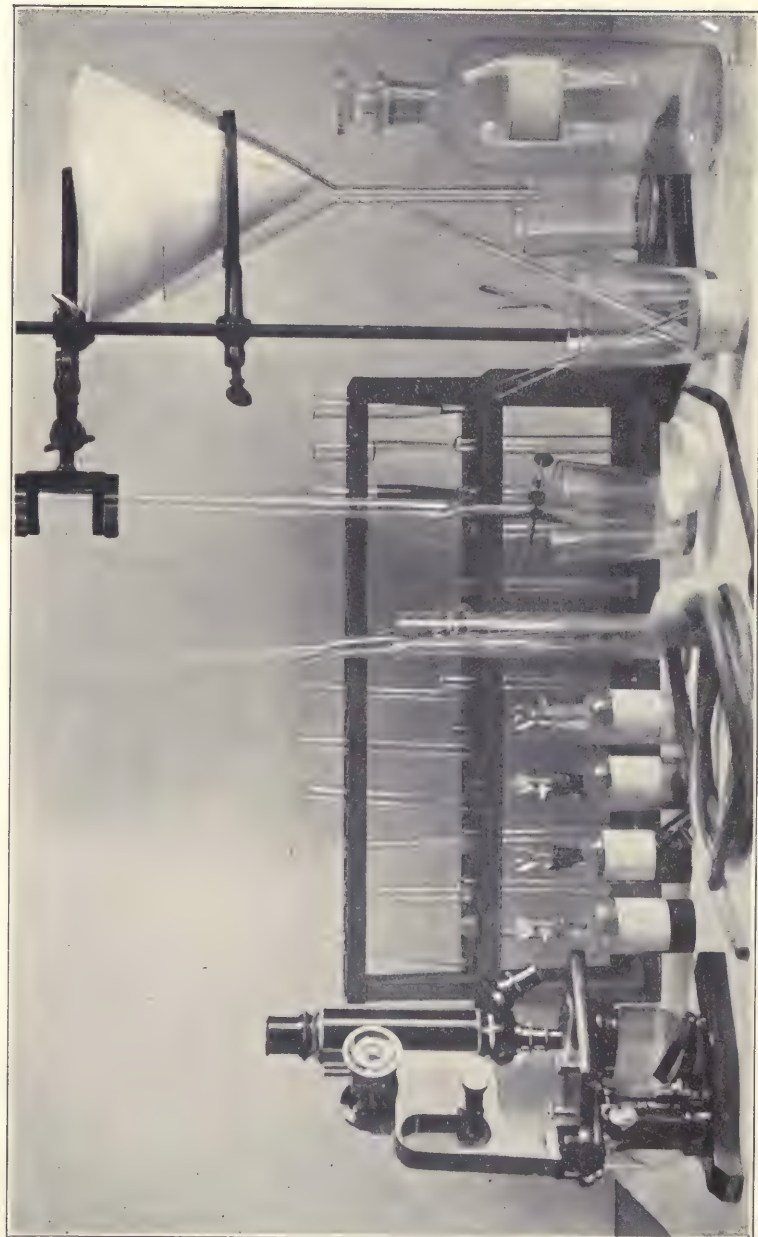


Fig. 19.—Apparatus for stomach analysis.

CHAPTER V.

CHEMISTRY AND BIOLOGY OF THE GASTRIC JUICE.

Apparatus.—Analysis of the gastric contents shows but few things of value to the clinician or the general practitioner, but these are very important in making a correct diagnosis of a stomach disease. In order to make these tests with ease and accuracy, only a few special pieces of apparatus and a few reagents are necessary :

- | | |
|--|---|
| 1. Buret, graduated to 50 cc., with stop-cock or pinch-cock. | 4. Phenolphthalein, a 1-percent alcoholic solution. |
| 2. Sodium hydrate, $\frac{1}{10}$ normal. In order to obtain dependable results, this solution must be accurate. Prepared quantitative solutions are obtainable from reputable chemical firms, or may be prepared by an expert pharmacist. | 5. Gunzburg's reagent. |
| 3. Dimethylamidoazobenzol, a weak aqueous solution. | 6. Ferric chlorid. |
| | 7. Stomach tube with bulb. |
| | 8. Evaporating dish. |
| | 9. Delicately graduated pipette, 10 cc. |
| | 10. Slides and cover glasses. |
| | 11. Microscope and accessories. |

Contraindications for Stomach Washing.—

1. Recent hemorrhage from the stomach.
2. Diseased esophagus, which may lead to perforation with the tube.
3. Aortic aneurism.
4. Marked arteriosclerosis.
5. Angina pectoris.
6. Other examinations may precede the passing of a stomach tube. Tabes dorsalis, tuberculosis, nephritis, and anemia may produce grave gastric symptoms. A detached tenth rib may point to a gastropexia, or certain stigmata and symptoms may indicate the presence of hysteria.

Preparation.—At the regular breakfast time for the patient he should be given an Ewald test meal consisting of two slices of white

bread, with the crust removed, without butter, and a glass of warm water or weak tea. The bread, which should be somewhat dry, must be well chewed, and not dipped into the liquid, but washed down with it. The contents are to be removed forty-five minutes after the meal.

Passing the Tube.—It is advisable to inform the patient that the operation will be unpleasant, but not painful. The tube should be warm, and should be lubricated with a very thin coat of glycerin. The patient should be seated in a chair with arms, so that he can grasp them with his hands, his head thrown well back, and his mouth wide open. Stand on the right side of the patient, with your left arm around his head, so that the left hand can be used to guide the tube as it is passed. Seize the tube about five inches from the tip, and place the tip, pointing downward, against the posterior pharyngeal wall, at the same time directing the patient to swallow. Then, holding the tube with the fingers of the left hand to keep it from being coughed out, push it steadily and rather rapidly down the esophagus. At the cardia it will meet with a little resistance, but this is soon overcome, and the tube will enter the stomach. If it meets a firm resistance, it is most probably against the stomach wall, and should be drawn back an inch or two. Direct the patient to close his lips and breathe rapidly, which will distract his attention and help to prevent retching. If the patient is very irritable or nervous, spray the pharynx with a weak cocain solution a few minutes before passing the tube. Generally, if care is taken and the operator does not use too much haste, no trouble will be found in passing the tube.

To Remove the Contents of the Stomach.—Grasp the tube with the right hand (Fig. 20), closing the tube at *A* with the thumb and forefinger of the same hand. Compress the bulb and close the tube at *B* with the fingers of the left hand, and immediately release at *A*. The bulb will expand, and the contents will be drawn through the tube into the bulb. Repeat this process until no more fluid is obtained.

If, after removing the contents for analysis, it is desirable to wash out the stomach for therapeutic purposes, the free end of the tube can be placed in a vessel of warm water, and the valve action of the fingers reversed so as to pump water into the stomach. In the same way the tube can be used as an air compressor to dilate the stomach, so that it can be outlined by percussion.

The tube is removed by a slow, steady pull. Give the patient a drink at once and food in about half an hour.

Examination of the Contents.—The material obtained should be examined as soon as possible after removal in order to avoid the artifacts of continued fermentation. Note the odor at once to determine if there has been any putrefaction. Filter the material through a single sheet of filter paper. Use a large funnel, as the filtrate will pass through slowly at best. Reserve some of the unfiltered liquid for microscopic examination.

Macroscopic Examination.—**QUANTITY.** This is a variable factor, and therefore no standard of normal can be given. It will vary with the motor activity of the stomach and with the amount

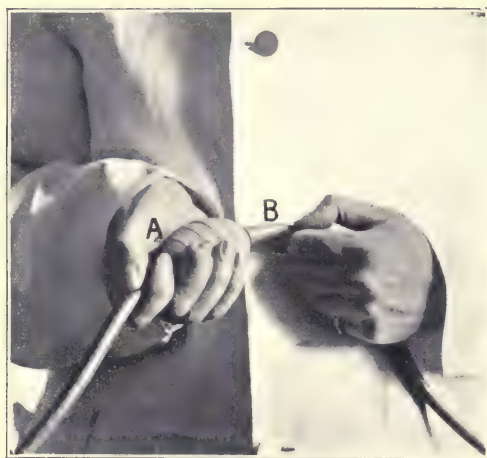


Fig. 20.—Method of removing contents from stomach.

of secretion. A large amount may signify retention or hypersecretion, or both, and a small amount may indicate hypermotility or scanty secretion. The chemical and microscopical examinations must be made as a check for the quantity of material.

COLOR. The normal color after an Ewald meal is yellowish-white. The presence of red blood indicates hemorrhage at the time of passing the tube, and may or may not have been caused by the tube, but is generally considered as a sign of congestion. Dark, brown and black specks or particles indicate hemorrhages previous to the passing of the tube, and are partially digested blood clots. A greenish-yellow tinge is caused by bile, which is often forced

back into the stomach by the straining and retching when the tube is passed.

PHYSICAL CHARACTERISTICS. The material in health consists chiefly of finely divided particles of the food taken. Mucus, of which there should be only a small amount, is recognized by its tenacious character. Food taken at a previous meal, or even a day or two before, can often be identified by bits of vegetable or meat fibers, seeds, and skins of fruits or vegetables. Of these the most easily recognized are the red skin and seeds of tomatoes and the skins of raisins.

Pieces of mucosa or tumor are difficult to recognize, and suspicious materials should be sectioned and examined histologically. (See *Essence of Tissue Diagnosis*, page 78.)

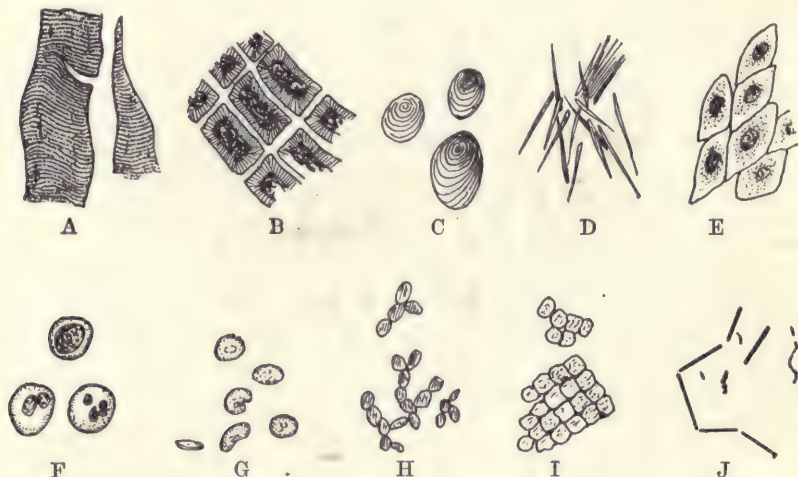


Fig. 21.—Microscopic elements of major and minor import in stomach analyses. Normal findings—A, muscle fibers from food; B, vegetable cells from food; C, starch grains of food; D, fatty needles from food; E, squamous epithelium from mucosa. Pathological elements—F, white blood cells, normal in small numbers; G, red blood cells, rarely normal even in very small numbers; H, yeast cells; I, sarcines; J, Oppler Boas bacilli, longer rods.

Microscopic Examination.—Take some of the material which remains in the filter, and press a small amount of it between two large glass slides or between two plates of clear glass. Examine it first with low power for connective tissue fibers and pieces of mucosa and tumors. Examine with high power for the following (Fig. 21) :

BLOOD CELLS. These may be in their normal shape or crenated.

YEASTS. These are elongated bodies, three or ten microns in length, with similar smaller processes budding out from them.

SARCINES. Sarcines are rather large cocci, which form "cotton bale" clusters as they divide.

OPPLER-BOAS BACILLI. These are long, heavy bacilli, which often occur in pairs, and may be found in long chains or forming angles with each other.

Chemical Analysis.—**QUALITATIVE.** *Free hydrochloric acid.* Mix 3 or 4 drops of Gunzburg's reagent and an equal amount of the filtrate on a porcelain shell or on the bottom of a clean evaporating dish, and heat slowly over a flame, holding the dish with the fingers in order not to burn the materials. A deposit of minute red crystals at the edge of the mixture gives a distinct rose-red color when free hydrochloric acid is present. This is a certain reaction.

Lactic acid. In a large test tube mix 5 cc. of the filtrate with 50 cc. of distilled water, and add 2 drops of ferric chlorid (5-per-cent aqueous solution). If lactic acid is present, a greenish-yellow color appears.

These two qualitative tests are the only ones of any value to the practitioner.

QUANTITATIVE. The most important quantitative analyses are for free hydrochloric acid and total acidity, and these may be made together. To 10 cc. of the filtrate in a beaker or whisky glass add 1 drop of dimethylamidoazobenzol indicator. Starting with the buret filled with the $N/10$ NaOH to the 0 cc. mark, add the NaOH to the filtrate, a drop at a time, stirring constantly. When the pink color has disappeared, all the free HCl has been neutralized, and the reading of the buret gives the number of cubic centimeters of NaOH used in the neutralizing. As only 10 cc. of filtrate were used, this number multiplied by 10 will give the percentage of HCl in 100 cc. of the filtrate in terms of $N/10$ NaOH. For example, if 4.5 cc. $N/10$ NaOH are required to neutralize the free HCl in 10 cc. of the stomach washings, then 10×4.5 , or 45, is the percentage of free HCl in terms of $N/10$ NaOH.

To the same filtrate now add 2 drops of phenolphthalein, and again titrate with NaOH until the first permanent pink occurs. The reading of the buret $\times 10$ now indicates the total acidity, and this, minus the free acid, is practically the amount of combined acid.

In case that enough filtrate can not be obtained to furnish 10 cc. for the quantitative tests, use as much as possible and adjust the figures according to the following example:

Number of cubic centimeters used : 10 :: NaOH used : x, x equaling the number of cubic centimeters of NaOH that would be required by 10 cc. of the filtrate.

If 6 cc. of the filtrate required 3 cc. NaOH for free HCl, then

$$6 : 10 :: 3 : x$$

$$x = 5$$

$$5 \times 10 = 50 = \text{percentage of free HCl.}$$

If 2 cc. more of NaOH are required for the total acidity, then

$$6 : 10 :: 5 : x$$

$$x = 8.33$$

$$8.33 \times 10 = 83.3 = \text{percentage of total acidity.}$$

$$83.3 - 50 = 33.3 = \text{percentage of combined acid.}$$

Interpretation of Findings.—*Yeasts* require time to grow, and may be considered as indicative of some retention and fermentation.

Sarcines can grow in free acid, and are generally indicative of hyperacidity.

Oppler-Boas bacilli are generally found in hypoacidity, as they do not grow well in free acid. They produce lactic acid, and where one is found the other may generally be expected.

In ulcer of the stomach the free and combined hydrochloric acids are increased, hemorrhage is common, and sarcines may be present. There is often a hypersecretion, so that large amounts of material may be obtained in washing. Retention will be shown. No lactic acid is found in the majority of cases.

Carcinoma of the stomach is accompanied by a diminished hydrochloric acid content, and often no acid is found. The Oppler-Boas bacillus is nearly always found, and is considered by some as pathognomonic of carcinoma. The secretion is scanty, and only small amounts of material are obtained after a test meal. Lactic acid is generally found.

Chronic gastritis gives a variety of findings, and the most constant of these is mucus in large amounts after a meal or after a fast of a few hours.

Gastric neuroses may be indicated by the presence of abnormally large or deficient quantities of stomach acids without the presence of pathological elements.

Sources of Error.—The main sources of error may be traced to inaccurate quantitative test solutions. When making the iron test for lactic acid it is advisable to run a control test, as the final green color is often difficult to determine. It may be very tedious to filter the sample, which is especially the case when much mucus is present, and it is suggested to pass it through a single piece of cheese cloth before using the paper.

Value and Limitations.—A thorough physical examination should precede every stomach analysis. If this fails to reveal the nature of the disorder, and there are no contraindications, the physician should proceed with the analysis of the gastric juice and expect to learn much from the examination.

It must be borne in mind that the results of a laboratory examination should not overshadow those obtained by other measures, and that each link in the chain of evidence must be equally strong in order that a safe diagnosis may be established.

Less Frequently Applied Procedures.—The stomach whistle, the gastric bucket, and mirror have yet to earn a place in the equipment of the general practitioner.

Many chemical and microscopic examinations of scientific interest have been omitted in order that the more useful procedures in diagnostics might be emphasized.

NOTE—Weinstein (Archiv. of Diag., July, 1912) calls attention to the fact that many valuable points may be gained by the gross appearance of the stomach contents. Thus, he states, chronic gastritis is characterized by thick contents with considerable amounts of stringy, glassy mucus. In the hyperacid states, the particles of bread are heavy; and fall to the bottom of the vessel like grains of sand. In hypoacidity, the particles of bread are more likely to be flaky and float.

CHAPTER VI.

ESSENCE OF TISSUE DIAGNOSIS.

The subject matter of this chapter has, for convenience, been treated under two divisions—"Essence of Frozen Sections" and "Essence of Celloidin Sections." The first division treats of the rapid tissue diagnosis in the operating room by means of frozen sections, but, as it is often desirable to proceed more slowly, the treatment in the second division offers a simple technic. Both methods have been used by the authors with the most gratifying results. Curettings from the cervix uteri, specimens from suspected breast tumors, and other tissues are examined by the first method and a diagnosis made within five minutes, provided that the technic has been thoroughly mastered by practice with pieces of steak, lumps of sausage, etc.

In this chapter no attempt is made to teach pathology, but the practitioner may, however, gain much information on this subject by frequent reference to the high-class atlas.

ESSENCE OF FROZEN SECTIONS.

Apparatus.—The articles forming the equipment have been listed in the order of their use.

- | | |
|--|--|
| 1. Whisky glass. | 10. Watch glass, or saucer. |
| 2. Formalin, 10-percent. | 11. Distilled water. |
| 3. Scalpel. | 12. Needle and section lifter, or substitutes. |
| 4. Forceps. | 13. Slides. |
| 5. Microtome. | 14. Thionin stain and pipette. |
| 6. Common bottle corks. | 15. Cover glass. |
| 7. Gum arabic (concentrated aqueous solution) and pipette. | 16. Filter paper. |
| 8. Tube of ethyl chlorid. | 17. Microscope and accessories. |
| 9. Section knife, or razor. | |

References.—Mallory and Wright: Pathological Technique; Dürck: Atlas of Pathologic Histology; Warthin: Laboratory Work in Pathology; Hall and Herxheimer: Pathological Methods.

With the exception of the microscope and small microtome, the entire outfit may be kept in a small box about 12x5x3 inches, which may be easily taken to the hospital or home of the patient. While quite as reliable results may be obtained with this outfit as with the more expensive apparatus, it must not be inferred that the specimens will be beautiful. The frozen section is, at best, thicker than the celloidin cutting, but, so far as diagnosis is concerned, shows quite as much.

Microtome.—A perfected microtome is a valuable, but expensive, instrument. The authors have tried many substitutes, and have found that a thin section, made with a razor, using a pair of tissue forceps and an ethyl chlorid spray, is satisfactory. Relihan suggests the use of a small rubber band wrapped around the points of

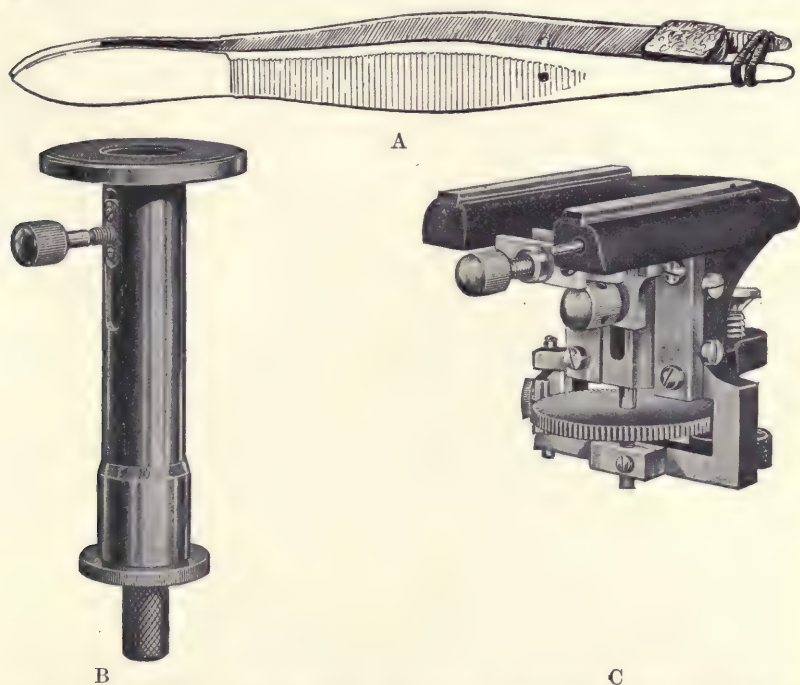


Fig. 22.—Substitutes for the perfected microtome. A, Relihan's suggestion, costs 10 cents; B, hand microtome, costs \$6; C, table microtome, costs \$12 to \$14.

the forceps (Fig. 22), the upper edges of the blades serving as ways and the razor being thus supported at a cutting angle. He says: "The first section will be too thick, but, after freezing the third or fourth time and repeating the same procedure, I have had no

trouble in getting thin sections. The contraction of the forceps blades seems to be so much quicker than the tissue contraction that the projecting portion is about the right thickness for a section. By going through this procedure . . . these sections answered my purpose perfectly."¹ The hand microtome (Fig. 22) answers for thick sections.

The authors recommend the use, whenever possible, of a table microtome, which is supplied by all optical firms, and with it practically every section is thin enough for study. Although the description is confined to this instrument, all directions may be so modified as to be applicable to any substitute.

Freezer.—Certain ether and rhigolene freezers, ranging in price from \$6 to \$10, are on the market and give good results. They do not, however, freeze under certain atmospheric conditions, nor are they easily manipulated when haste is desired. The ethyl chlorid spray, used in minor surgery, is more convenient, can be had at a modest price, and the results of its use are so encouraging that it is recommended to the general practitioner.

Knife.—Any razor will answer, especially if one side of the blade is flat—not hollow ground. The authors have successfully used the new Bausch & Lomb chisel knife, an inexpensive instrument, for frozen sections.

Fixing and Mounting.—With the scalpel and tissue forceps the specimen is cut into a small block about the size of a pea and dropped immediately into a tumbler containing 10-percent formalin (4-percent formaldehyd), when it is carried to where the examination is to be made, and the short time that it is in the solution will aid in freezing it. Alcohol delays freezing and does not answer the purpose. The tissue is then seized with the forceps and dipped into a concentrated aqueous solution of acacia, after which it is placed on the top of an ordinary cork previously mounted on the microtome.

Freezing and Sectioning.—The tissue, in its gummy capsule, is frozen at once by using the ethyl chlorid spray. Very little of this liquid is necessary if the operator blows vigorously on the tissue, and under such treatment it should harden almost instantly. Sections are quickly obtained, and should be as thin as possible.

Staining.—The sections are dropped immediately into some dis-

¹ Journal of American Medical Association, November 6, 1909.

tilled water, which should not be too cold if good stains are desired. Place a section on a clean slide and add immediately several drops of carbol-thionin. Permit staining to proceed for at least one minute, and take up excess of fluid with a little filter paper. Add a clean cover glass and examine under low power.

Preparing the Stain.—The stain is so easily made up that the powdered thionin is recommended. The making of the liquid stain, as described by Strouse, is as follows:

1. Take 2 grams of pure thionin.
2. Make up a saturated aqueous solution.
3. Allow the solution to stand at least six hours, stirring occasionally.
4. Filter.
5. Mix the clear solution with equal parts of 2-percent phenol.

The stain improves with age, but an occasional filtering may be necessary.

Technic.—A brief outline of the technic of quick tissue diagnosis is as follows:

1. Cut into small block.
2. Transfer in 10-percent formalin.
3. Mount on cork in acacia solution.
4. Freeze with ethyl chlorid.
5. Section.
6. Float in warm distilled water.
7. Drop on slide.
8. Add stain.
9. Add cover glass.
10. Examine.

Examination of Section.—Too much must not be expected from this method, and even a person with some experience in the ordinary technic may be confused at first. Compared with celloidin sections, these are much thicker and do not stain so well. With a little patience, however, and when applied to certain selected cases, it proves a very valuable addition to the medical laboratory. Examinations should be made principally with a low-power objective, especially in diagnosing tumors. The thionin stain is usually not intense, so that it may be advantageous to narrow the diaphragm. Nuclei are stained a dark-blue color, while the protoplasm takes on a reddish purple tinge.

Errors in Technic.—Alcohol can not be substituted for formalin,

as it prevents or delays freezing. The refrigeration must be complete, and the tissue should, figuratively speaking, become as hard as a rock before any attempt at sectioning is undertaken. At the first indication of thawing, the tissue should be again frozen if more sections are desired. A dull knife and a straight cut cause the tissue to crumble. The knife should be drawn at an angle best estimated by experience. Staining should cause no trouble, and a thionin solution which refuses to stain after it has been prepared two weeks should be rejected. Neither the stain nor the wash which precedes it should be cold if good results are desired.

Other Freezing Methods.—In hospitals and other institutions, where expense must serve convenience, the carbon dioxid freezing tank and attachment are used.

Value and Limitations.—The freezing methods are used at present only in these diagnostic indications which have been previously considered (page 78), and are not advised where haste is not necessary, as the sections are thick, stain poorly, and are often of no service in the differentiation of cellular elements. In its sphere, however, the value of the rapid method can hardly be overestimated. The technic can not be mastered in a few minutes. Repeated attempts with controls—tissues of known sources—should be made, and the apparatus collected and held in reserve for the time of need.

ESSENCE OF CELLOIDIN SECTIONS.

Apparatus.—

- | | |
|--------------------------------|--|
| 1. Absolute alcohol. | 9. Glass covers. |
| 2. Blotter system. | 10. Mayer's hemalum solution. |
| 3. Carbol-xylol. | 11. Microscope and accessories. |
| 4. Celloidin shavings. | 12. Microtome and knife, or substitutes. |
| 5. Corks. | 13. Section lifter. |
| 6. Distilled water. | 14. Staining dishes, or substitutes. |
| 7. Eosin (1-percent solution). | 15. Whisky glasses. |
| 8. Ether. | |

Of all the celloidin methods, this seems to be the most simple and rapid, as well as sufficiently accurate, technic for those desiring to do this kind of work.

Preparing Celloidin Solutions.—Shering's celloidin shavings are sold in 1-ounce bottles by optical firms. A stock solution is prepared by dissolving about 2 drams of the shavings in a 6-ounce

bottle filled with equal parts of absolute alcohol and ether. Keep well stoppered, shaking occasionally, and a perfect solution should occur within twenty-four hours. This is the stock solution, or the thick celloidin solution. To prepare the thin celloidin solution, dilute some of the stock solution with an equal quantity of a solution of equal parts of absolute alcohol and ether.

All of these solutions are very volatile and highly inflammable, and must be kept in well-stoppered bottles. When used in glasses or staining dishes, the vessels should be covered with small squares of window glass, similar to those used when examining urine sediments. In case the solution must stand over night, a little petrolatum should be placed around the upper edge of the vessel before applying the cover.

Rapid Hardening and Infiltration.—This method, somewhat modified, is used in the pathological laboratory at the University of Michigan. Each solution may be kept in a small tumbler, but evaporation must be avoided. A piece of the tissue about the size of a pea is selected and passed through the solutions as follows:

1. Three changes of absolute alcohol during an hour.
2. Equal parts of ether and absolute alcohol, one-half hour.
3. Thin celloidin over night.

Imbedding.—Remove the tissue from the thin celloidin and place it on the flat surface of a wide, but not too thick, cork—a cork similar to those used in small salt-mouthed bottles. Permit the celloidin to partially “set,” thus gluing the preparation to the cork, and then pour on it a little thin celloidin and blow on it. By repeating this process several times the celloidin may be built up around the tissue. Now float the cork, tissue face downward, in absolute alcohol for five hours or longer.

Sectioning.—(See page 80.) Do not use the chisel knife, but an ordinary microtome knife or a razor. In case the latter is employed, at least the under surface should be flat, and the upper surface must be kept covered with alcohol during the cutting. The section should be made by one continuous cut, using a good “cutting angle.” Sections are placed in ordinary alcohol before staining.

Systematic Staining (Authors' Method).—By using picrocarmin, less time will be taken, but the results are not nearly so good as where hemalum and eosin are used. Instead of staining dishes, watch glasses or saucers may be used. Small tin ointment

boxes possess many advantages, and, if they are new, the sections are easily seen against the bright tin background. Before beginning any portion of the staining, each dish should be supplied with its solution, covered with a glass plate, and be arranged in the order of their use. A watch or clock should be constantly in full view. The plan of systematizing here given has many advantages over the more haphazard methods.

Each dish should rest on a small piece of blotter, which not only serves to keep the laboratory table clean, but on it may be placed the number of the dish, the name of the solution, and the staining time. This arrangement will not only keep the worker from becoming confused, but, if laid aside between analyses, it will not always be necessary to read up the method when each tissue is examined, which is of importance to the busy practitioner. The following procedure not only illustrates what is meant by the "systematic" method, but serves to describe the routine of celloidin staining as recommended to the general practitioner.

1. Hemalum, one to ten minutes—nuclear stain.
2. Distilled water, one minute—wash.
3. Eosin, one to five minutes—cytoplasm stain.
4. Distilled water, one minute—wash.
5. Ninety-five-percent alcohol, one minute—dehydrating.
6. Absolute alcohol, one minute—dehydrating.
7. Carbol-xylol, one minute—clearing.
8. Mount in balsam.

The duration of staining depends on the strength of stain and kind of tissues. With a little practice this method should give no trouble. The nuclei should appear as dark-purple spots on a pink cytoplasm. In case it is not desired to keep the specimens, the technic may be stopped short of the alcohols and the examination be made in water.

Curetings.—These may be prepared for examination by either the freezing or celloidin method, several pieces, instead of one, being mounted in acacia or imbedded in celloidin respectively.

Paraffin Imbedding.—A description of this method is left to the larger text books. It is true that very beautiful specimens may be obtained by the use of this substance, but for a small number of specimens it can hardly be recommended. Its application necessitates the purchase of expensive equipment, and it is a slow method so far as diagnosis is concerned.

CHAPTER VII.

DETECTION OF THE COMMON POISONS.

Apparatus.—

1. Ring stand.
2. Evaporating dish, or porous porcelain plate.
3. Marsh apparatus.
4. Test tubes, stand, and holder.
5. Funnel and filter paper.
6. Reagents, which should be chemically pure and obtained in small amounts from reliable companies—carbolic acid, chromic acid, hydrochloric acid (arsenic free),

nitric acid, sulphuric acid, ammonium hydroxid, calcium chlorid, powdered charcoal (wood), ferric chlorid, Nessler's reagent, phenolphthalein (1-percent alcoholic solution), potassium bichromate (dichromate), sodium hypochlorite (should be made up fresh by pharmacist), stannous chlorid, sweet oil, zinc (arsenic free).

In this book are described only the good tests, which are usually the simple ones, and, with a view of limiting expense, the selections of tests have been made in such manner that one reagent may be available for the detection of several different drugs. For example, the ferric chlorid can be used in the identification of morphin, opium, and carbolic acid, and the same reagent may be used in urinalysis. As far as possible the authors have avoided indicating the rare and more costly reagents.

These are sample tests, and are made for the purpose of satisfying the physician or his patient. Only an isolation of the poison in its pure form is accepted as expert testimony, and it is not the object of this book to enter that field.

There are certain limitations to the value of many of these tests, but these are not always pointed out because substances giving similar reactions are not usually met under the same conditions. For example, the physician observes certain symptoms in a patient which he believes to be due to phenol poisoning, and at once begins examining samples of the drug, food, or beverage under suspicion. The fact that anilin might give a similar reaction seems hardly to

References.—Peterson and Haines: Legal Medicine and Toxicology; Edmunds and Cushny: Experimental Pharmacology; Autenrieth: Detection of Poisons; Riley: Toxicology; Tanner: Poisons.

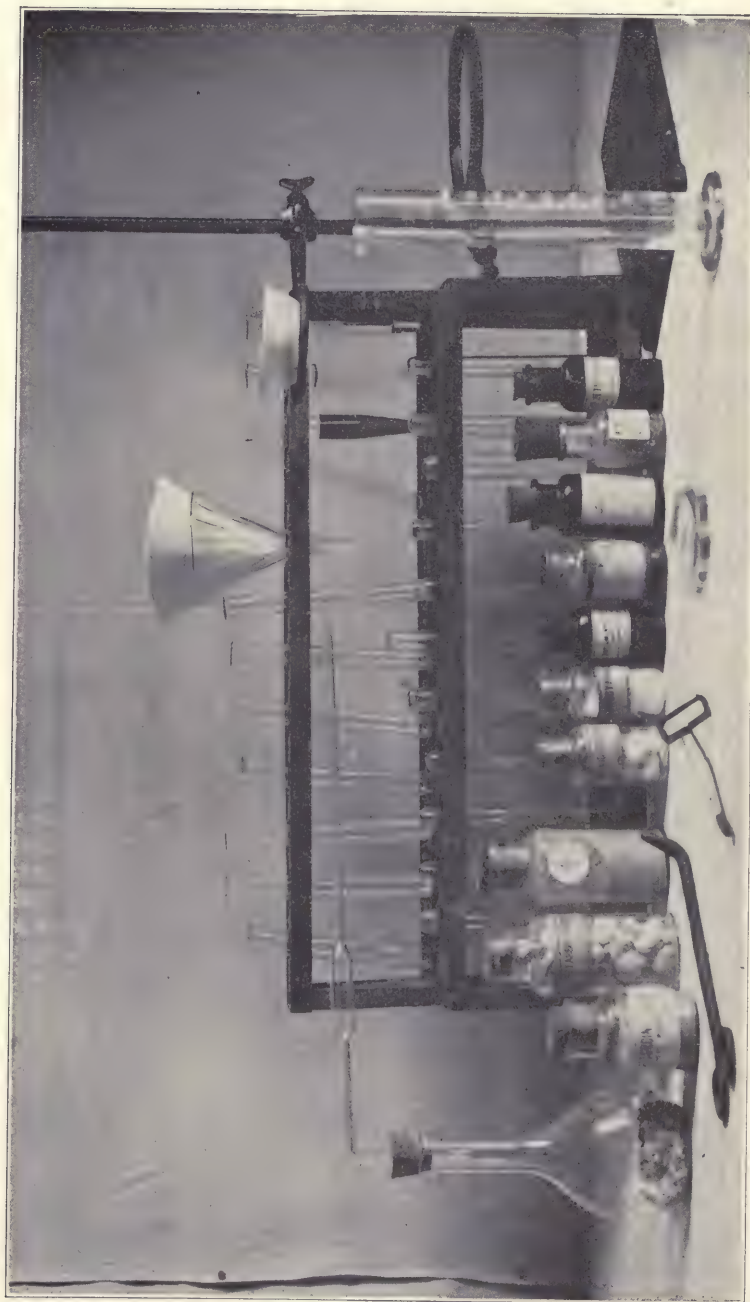


Fig. 23.—Apparatus for detection of common poisons.

enter into the question. So far as the **common poisons** are concerned, **each reaction is specific**, and should at once put the physician on the right track, but such conclusion would not be accepted in a court as expert evidence. While these reagents are few and not expensive, they are not always to be obtained by the physician at a moment's notice, and, if he has a considerable amount of this work to do, he should keep on hand a sufficient quantity to meet his needs.

All poisons are not freely soluble, so that the sample should not be filtered. Much better results may be obtained by straining out the larger pieces of foreign material, and decantation may often serve this purpose. Only the most common poisons are included, and it has been deemed advisable to omit aconite and other poisons of perhaps secondary import for the reason that they are not usually employed to destroy human life. For the sake of simplicity, names of the tests are not given, as many have been modified to such an extent that it would be difficult to give a definite nomenclature.

In the poison tests distilled water should, if possible, be used, although it is not absolutely necessary. In case ordinary water is used, it should contain neither lead nor any other common poisons—in other words, it should be good drinking water.

Unfortunately there are no simple tests for wood alcohol and antipyrin which can be properly recommended, while several other tests—for example, the ergot test—are none too satisfactory.

Sources of Every-Day Poisons.—"Dopes" are neither always served "straight," nor are they invariably so preferred by suicides. Some of the more common mixtures or adulterations are:

Abortifacients—ergot, yellow phosphorus.

Bad whisky ("rot gut")—laudanum, wood alcohol.

Bitters (bracers)—strychnin, alcohol in large proportion.

Bug poisons—arsenic, mercury salts.

Coke, coc, certain colas, fatigue powders—cocain.

Cough syrups, soothing syrups, papine—morphin.

Drug cures—morphin, cocain, alcohol, hyoscin, etc.

Eye drops, clap medicines—zinc salts, silver salts.

Headache powders—acetanilid, antipyrin.

Knock-out drops—chloral, laudanum.

Liniments—certain ones have been found to contain wood alcohol.

Lye—fixed alkalies.

Matches—yellow phosphorus (some of the safety matches contain the nonpoisonous red phosphorus).

Paris green—a mixture of copper acetate and copper arsenite.

Rat poisons—arsenic; some contain phosphorus, others contain cultures of the Danyez virus or certain strains of paratyphoid bacilli.

Sex stimulants—cantharidin.

Skin “beautifiers”—arsenic.

Inasmuch as many poisons have been omitted, there has been no attempt at classification other than an alphabetical arrangement. For convenience, the names of necessary reagents precede the test. The letter “x” refers to the unknown substance.

It is not necessary that percentage solutions should cause confusion. In qualitative tests, only approximate values are intended unless otherwise stated. A practical method of estimating these solutions is given in General Information, page 192.

Acetanilid.—*Concentrated hydrochloric acid, concentrated aqueous solution of carbolic acid, ammonium hydroxid solution, very dilute chromic acid solution.* Boil “x” with about $\frac{1}{2}$ dram of concentrated hydrochloric acid and cool. Add 1 dram of the carbolic solution, and then add a few drops of the chromic solution. After two minutes a dirty purple or red should appear. Add a few drops of the ammonium hydroxid solution and shake. Within two minutes a greenish or indigo color should appear.

Alkalies Versus Mineral Acids.—*Phenolphthalein (1-percent alcoholic solution).*

1. Alkalies have a soapy “feel.”
2. Nitric and hydrochloric acids have characteristic odors.
3. Acids act on carbonates with gas formation.
4. Acids show a tendency to exhibit a sour taste even in very dilute solution.
5. Test with phenolphthalein. Alkalies in very small amounts impart to this indicator a beautiful red color, whereas acids produce no effects.

Alcohol.—*Sulphuric acid (50-percent), potassium bichromate.* Add to $\frac{1}{2}$ dram of “x” $\frac{1}{4}$ dram of the acid, and drop into the mixture a crystal of the bichromate. A green color should appear.

Ammonia.—*Hydrochloric acid.* Dip a stirring rod into the acid and hold near “x.” White fumes should appear. The charac-

teristic odor of ammonia as well as the gas is lost on standing. Samples for examination should be tightly corked.

Arsenic.—*Hydrochloric acid, zinc, sodium hypochlorite, calcium chlorid.* Mix "x," 4 drams of hydrochloric acid, several pieces of zinc, and 1 ounce of water (distilled or drinking) in a small Marsh apparatus, and cork. Do not inhale fumes. Do not light gas until you are sure all air has been expelled from the bottle. Test by collecting samples in inverted bottle, and, when these will burn without explosive violence, all air has been removed.

Fig. 24 shows how a Marsh apparatus may be constructed with a bottle, a cork, and a graduated 5-cc. pipette, and illustrates how the hydrogen must be collected for testing. There must be no leaks in the apparatus. Now **carefully** light the gas, as, in case any air is present, a violent explosion will occur. Be careful to inhale none of the fumes, which give off a garlic-like odor and are very poisonous. Hold one of the unglazed porcelain plates in the flame, and, if a sublimate is deposited which is

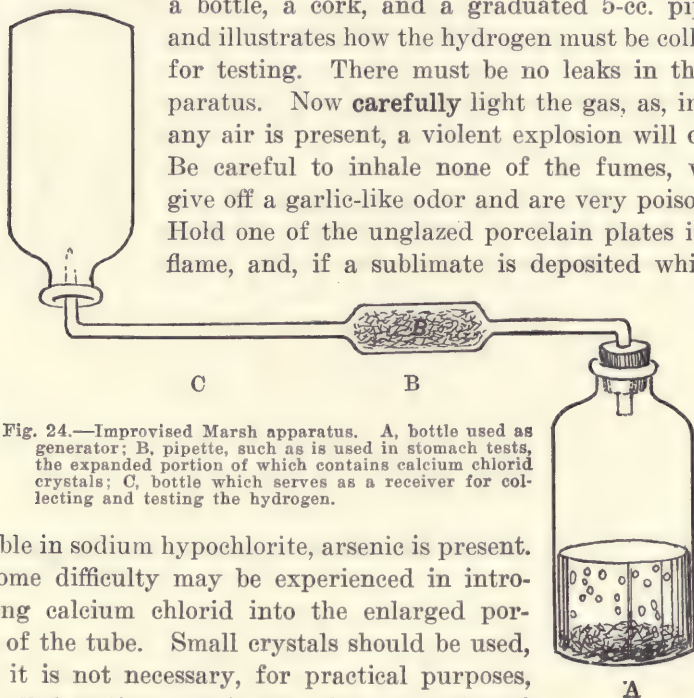


Fig. 24.—Improved Marsh apparatus. A, bottle used as generator; B, pipette, such as is used in stomach tests, the expanded portion of which contains calcium chlorid crystals; C, bottle which serves as a receiver for collecting and testing the hydrogen.

soluble in sodium hypochlorite, arsenic is present.

Some difficulty may be experienced in introducing calcium chlorid into the enlarged portion of the tube. Small crystals should be used, and it is not necessary, for practical purposes, that all these lie neatly in the enlarged portion of the tube, nor is it imperative that this enlargement should be entirely filled. If a thistle tube can be obtained and passed through the cork, its lower end diving well below the liquid, fresh acid may be added from time to time as necessary. The amounts of reagents need not be accurate, and necessarily vary with the size of the bottle. When cleaning apparatus, avoid inhaling odors. Throw away the zinc.

The following instructions should be observed.

1. Have all connections tight.
2. Do not inhale fumes.
3. Do not explode your apparatus.
4. When lighting the gas, use a long rod to support match in order to guard against injury if an explosion should occur.

Atropin, Hyoscin, Etc.—Both dilate the pupil of a dog's eye. When a sample of atropin is heated, a honey-like odor is given off, which may be intensified by the addition of oxidizing agents.

Bacteria.—It seems that the future toxicologist must be trained to recognize certain pathogenic bacteria, especially the typhoid bacillus. Their isolation and identification are tedious, discouraging, and often impossible, even for the expert. The physician should bear in mind this new method of voluntary contamination of food and prescriptions.

Cantharidin.—*Sweet oil.* Dissolve some of the suspected material in hot sweet oil, and shake well. When the oil rises to the top of the mixture, decant it into a separate vessel. Apply several drops to some adhesive plaster and place it on the chest, allowing it to remain in position about ten hours. A positive test is shown by the presence of a blister, or at least a severe reddening, where each drop touches the integument. Alkalies, mineral acids, and other poisons which might give a similar test are identified in a different manner.

Carbolic Acid.—*Dilute ferric chlorid solution, dilute hydrochloric acid.* Add to "x" a few drops of the ferric chlorid solution, when a green, blue, or violet color should appear, depending on the amount of the poison present. Then add a few drops of the hydrochloric acid, when a lemon-yellow color will take the place of the blue. Alcohol interferes with this test. Phenol, even in very small amounts, has a characteristic odor.

Chloral Hydrate.—*Nessler's reagent.* A few drops of Nessler's reagent added to a chloral hydrate solution produces a precipitate resembling red brick dust, and later this assumes a green color. The odor of chloral hydrate is characteristic, reminding one of the odor of the American green walnut. Chloral fiends are sometimes diagnosed as diabetes patients, as the urochloralic acid of the urine reduces alkaline copper solutions.

Cocain.—*Aqueous solution of chromic acid (5-percent), concentrated hydrochloric acid.* To "x" add a few drops of the chromic

acid solution. Each drop will produce a precipitate, which will immediately disappear if the solution is shaken. Then add to this clear solution $\frac{1}{2}$ dram of the hydrochloric acid. A delicate orange-yellow precipitate indicates cocain.

Copper.—All copper solutions are not blue or green, as is the common belief. If, however, clean platinum wire is moistened with such solution and held in the colorless flame, the latter will become intensely green.

Ergot.—The chemistry of ergot is still unsatisfactory. Feeding ergot to chickens causes gangrene of their combs, but this method is of little value when a quick diagnosis is desired.

Formalin.—In amount sufficient for suicidal purposes, the odor is too characteristic to be mistaken, and experimental evidence shows that a large amount is necessary to kill. For its detection in milk see *Milk and Its Home Modifications*, page 134.

Hydrocyanic Acid.—The odor of hydrocyanic acid resembles that of peach blossoms, and is too characteristic to render necessary further means for identification. The poison is very volatile, and must be tested at the earliest moment. It should not be inhaled in appreciable quantities.

Lead Acetate (Authors' Method).—*Dilute nitric acid, concentrated sulphuric acid.* Sugar of lead has a sweetish, astringent taste. It shows a tendency to form a milky solution, and contains undissolved crystals. Add just enough of the dilute nitric acid to clear the cloudy solution, and then add 1 or 2 drops of the concentrated sulphuric acid. The heavy white precipitate is lead sulphate.

In case "x" can not be cleared by a few drops of the dilute nitric acid, add 1 drop of the latter to another sample of the unknown, filter, and test this filtrate with the sulphuric acid.

Barium and strontium may respond to the same test, but usually need not be considered. In case their presence is possible, barium will impart to the colorless flame a yellowish green and strontium will show a brilliant red.

Lead in Drinking Water.—(See *Some Simple Water Analyses*, page 140.)

Mercury Salts.—*Aqueous stannous chlorid solution.* Add to "x" some of this reagent. A gray and white precipitate indicates mercury, being a mixture of quicksilver and calomel. The test depends on precipitate formation. Many of these precipitates are

alcohol soluble, and one should be mindful of the possible presence of alcohol in all suspected material. For example, to prove that the stomach washings of a drunkard contained corrosive sublimate, it might be necessary to heat them slightly to cause the evaporation of the alcohol, or else to add an excess of the tin solution.

Morphin and Opium.—*Ferric chlorid solution.* Add to "x" a few drops of this reagent. If the color is obscured by a precipitate, filter it. A deep-blue color indicates pure morphin, while the presence of opium is shown by a beautiful dark-red. In case there is any doubt, compare with a filtered solution of "x," with the reagent, or with controls.

Phosphorus.—Suicide with match heads has become obsolete. At least four are necessary to produce death in an adult, unless oils or fats have been taken. The odor and phosphorescence in the dark are characteristic. It has been claimed that some rat poisons contain phosphorus in addition to arsenic.

Silver Nitrate.—This turns black when exposed to light. It is precipitated from its solution by common salt as insoluble silver chlorid. Lead or mercury may answer this test, but either may be otherwise identified and differentiated.

Strychnin.—*Concentrated sulphuric acid, crystal of potassium bichromate.* On a white background—a piece of paper—lay a clean slide. On this dissolve some of "x" in 2 drops of concentrated sulphuric acid, and in this mixture crush with a glass rod a crystal of potassium dichromate. A beautiful violet or blue color indicates strychnin. Very small amounts of the drug are bitter to the taste. Some of the sample injected with a hypodermic needle into a frog or other animal may bring on the typical tonic convulsions.

Sulphonal, Trional, Etc.—Either drug heated in a dry test tube with powdered wood charcoal develops a characteristic odor—ethyl mercaptan. If this odor is not familiar to the physician, he should run a control test, using sulphonal instead of "x."

Less Frequently Applied Tests.—Other substances, the descriptions of whose detection have been left to larger books, may be named as follows: anilin compounds, nicotin, aconite, veratrin, codein, oxalic acid, santonin, so-called ptomain poisonings, etc.

Difficulties and How to Avoid Them.—One of the most common causes of poor results in chemical analyses is working with the wrong reagent, and this is not always the fault of the worker.

Too many registered pharmacists have forgotten their chemistry, and examination of certain solutions supposed to be standardized by an expert pharmacist showed that no such work had been done. One of the authors was unable to obtain the diazo test in four cases which he knew to be typhoid, and an examination of his sulphanilic solution showed a 50-percent sulphuric acid. Too much must not be taken for granted.

Nomenclature is often confusing. Neither chromic acid nor carbolic acid is properly named. Glycerin is constantly referred to by some druggists as a sugar, but is really an alcohol. Quicksilver contains no silver, but is metallic mercury. These terms have not, however, been avoided in this book, as their use is too widespread.

A case is known where, in testing for lead by the iodides, a worker, usually careful, thoughtlessly employed lead iodid. For cautions against explosives and incompatibilities, see *Laboratory Prophylaxis*, page 180.

It is impossible in a book of this scope to teach the principles of chemical analysis, but the following general rules may prove of value to the man expecting good results:

1. Absolute cleanliness. Test tubes can not be kept too clean. Sugar of lead is especially prone to adhere to glassware. Do not touch with your fingers chemically pure reagents, but pour directly from their bottles into solvent or on clean paper, and then cork the bottle tightly. Never lay a cork or stopper on a table, but hold between fingers until it can be replaced.

2. Allow time for a reaction to take place before adding more reagent or deciding on a negative result. It can not always be explained why the same test may at different times employ more time.

3. If precipitates interfere with a color reaction, filter and examine the filtrate.

4. Before performing the Marsh test, study well the properties of hydrogen, how it is generated, and how handled.

5. Unless a worker is certain of his technic, a control test should be used in connection with each investigation. Many shortcomings may thus be detected which would otherwise escape observation.

Value and Limitation of These Investigations.—These have been pointed out in the matter preceding the tests.

CHAPTER VIII.

EXUDATES IN BRIEF.

Apparatus.—

- | | |
|------------------------|--------------------------------|
| 1. Centrifuge. | 5. Glycerin. |
| 2. Eosin. | 6. Microscope and accessories. |
| 3. Exploratory needle. | 7. Potassium hydrate. |
| 4. Formalin. | 8. Sodium citrate. |

Obtaining an Exudate.—An ordinary hypodermic outfit may sometimes be used, but it is advisable to employ a long and stout exploratory needle. All apparatus should be sterile, especially if any cultures are attempted. A small amount of the fluid suffices for the tests described in this book, but, as larger amounts are often removed for therapeutic purposes, it would not be a waste of time to take the specific gravity, or even attempt other examinations described in the larger volumes. A bacteriological culture may be easily made, but usually is not necessary for safe conclusions. If apparatus is sterile, any germ may be looked upon with suspicion, but the authors have often been able to gain more from a study of the cells than from a search for specific microorganisms. A safer plan is to investigate both. An ethyl chlorid spray will render these operations painless.

Thoracic Puncture.—The spot selected for pleuritic effusions is marked by the junction of the axillary line with the seventh intercostal space, and is clearly shown in Fig. 25. The arm is raised and the hand placed on the opposite shoulder in order to widen the interspaces. The relation of the intercostal artery to the lower edge of the rib must be kept in mind. Pericardial puncture is rarely attempted outside of the hospital. The technic may be found in the larger books.

Abdominal Puncture.—The spot varies, but is usually low. A short needle is desirable, so that the bowels may not be punctured.

Lumbar Puncture.—A short, strong sterilized needle is employed,

References.—Gruner: *Puncture Fluids*; all works on clinical diagnosis, including Sahli, Wood, Boston, Simon, etc.

but no syringe should be used unless its plunger has been previously drawn. The patient should lie on the left side, with the thighs flexed and shoulders bent forward. A line joining the iliac crests passes between the third and fourth lumbar vertebra. Select a

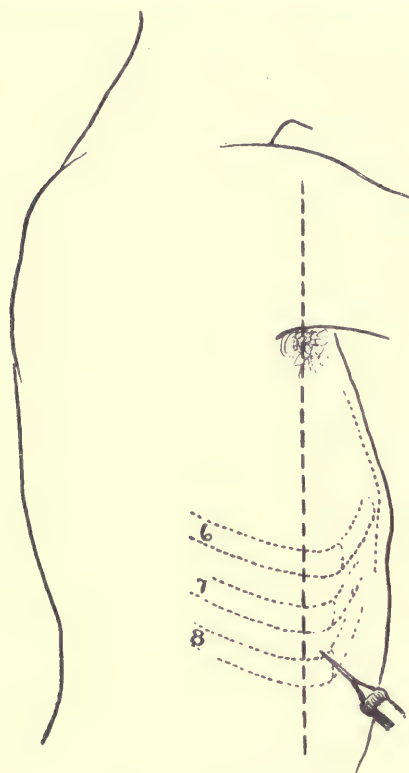


Fig. 25.—Puncture of pleura.

point midway between the spinous processes of these two bones, and place the point of the needle one-third of an inch to the left of this point and insert. Fig. 26 illustrates the exact position. Allow the liquid to flow as it will, and **do not aspirate**. If fluid does not come, have the patient strain, and it may be necessary to again insert the needle. The operator should be certain that the needle is not clogged before attempting this procedure. In these punctures, as well as all others including thoracic, abdominal, joints, etc., several precautions are emphasized:

1. All apparatus must be sterile.

2. Avoid important structures, as nerves, vessels, intestines, bladder, heart, etc.

3. Do not aspirate in lumbar punctures. If necessary to carry out this procedure elsewhere, do so very slowly and then permit excess to drain.

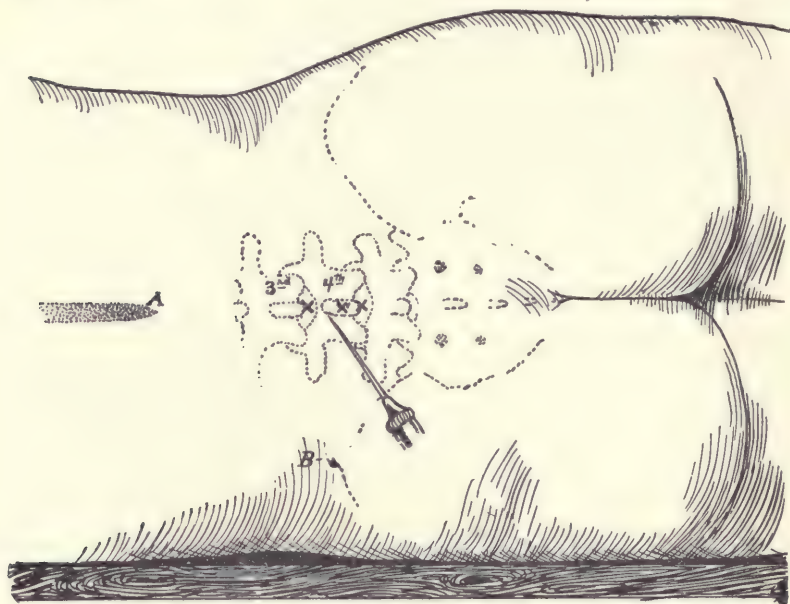


Fig. 26.—Diagram showing site of lumbar puncture. x,x, tips of spinous processes; x', point midway between; A, lower tip of spinal cord; B, ilium.

4. If patient shows any signs of collapse, stop the operation at once. Very often these punctures may be followed by a prompt relief of symptoms.

Preparing an Exudate.—As cells and bacteria may be difficult to find, it is usually advantageous to centrifugalize as with urine. Many of these exudates show a tendency, however, to clot very quickly, and to avoid this the tube may be one-quarter filled with 2-percent sodium citrate solution. The following process may then be followed:

1. Centrifugalize sufficiently.
2. Remove with a pipette the supernatant citrate solution.
3. Add an equal amount of dilute formalin, 1-percent aqueous solution.

4. Mix thoroughly by shaking.
5. Permit to stand five minutes.
6. Centrifugalize sufficiently.
7. Remove sediment from beneath liquid by the method described for urinary sediments. (See *The Urine in Disease*, page 116.)

8. Make spreads, dry, and fix by heat; stain with methylene blue.

Most Important Findings.—Various chemical tests can hardly be considered here. The cytoanalysis is doubtless the most important, and may be briefly given as follows:

1. Tuberculosis is characterized by lymphocytes and few red cells.

2. Tuberculosis with secondary purulent infection is characterized by a mixture of lymphocytes, polymorphonuclear leukocytes, and red cells.

3. An infection never tuberculous, but caused by the so-called purulent germs—as gonococcus, meningococcus, pneumococcus, streptococcus, colon or typhoid bacilli, etc.—is characterized by absence of red cells and lymphocytes, but large numbers of polymorphonuclear leukocytes are present.

4. Ascites, etc., contains a few mesothelial cells—large flat cells, with single nuclei.

Searches may be made for the various microorganisms. In case fluid from the lumbar puncture shows lymphocytosis, especially without red blood cells, the process may be syphilitic instead of tuberculous. Age, history, and other factors must be taken into consideration. If, however, it contains only polymorphonuclear leukocytes, and these in considerable numbers, it may be a meningococcus infection; in fact, this organism may be detected within the pus cells. Meningitis of an acute type has been caused by other germs, notably the pneumococcus, gonococcus, and typhoid bacillus. A few white cells may be found in a normal serous fluid.

Less Frequently Applied Procedures.—The practitioner does not usually attempt the diagnosis of general paresis by lumbar punctures. If he desires to “tap” the pericardial cavity, complete descriptions of this procedure may be found in the larger books. Fluids from ovarian cysts, spermatocele, etc., offer little to the diagnostician.

AzoöspERMATISM.—Family sterility is due in about two-fifths of all cases to lack of spermatozoa in the male. The condition of azo-

öspERMATISM can not be diagnosticated by a single examination, or by less than a dozen such investigations, to be made monthly. Spermatozoa may be absent from the semen during certain diseases. The frequent exposure to x-rays may cause a destruction of all spermatozoa and result in a permanent sterility. Spermatozoa may be present, but may be dead at the time of emission, falling a prey within the male to the treponema or other injurious influences. Such germ cells are not motile, but, in order to prove that loss of motion is not due to chilling, examination must be made at once, using warmed slides and cover glasses.

Spermatorrhea.—The male germ cells may be constantly present in the urine of many men, and is caused by certain sexual excesses, masturbation, and severe diseases. The urine of the female may contain spermatozoa, which exist as a vaginal contamination after intercourse.

Proof of Rape.—So far as legal processes are concerned, such questions are left to experts. The parents of the girl may, however, desire a private opinion, and they may not wish to subject the girl to an examination, but may bring some vaginal secretion or scrapings, which may be examined at once for spermatozoa; or, more commonly, a dry and stained cloth may be submitted. In case the semen was pure and is contaminated by little of the vaginal secretion, the stain will be almost colorless, and will give the cloth a “feel” not unlike that of starched, but unironed, linen. The characteristic odor may be present.

Place a small piece of the stained cloth in a few drops of a 1-percent aqueous solution of caustic potash, and allow to soak for at least two hours. Pour off liquid and add several drops of glycerin. Tease thoroughly, but gently, as the tails are very easily broken. Add 1 drop of eosin and mix thoroughly. Examine some of the liquid under oil immersion lens. The spermatozoa are stained red, but cotton and linen fibers are unstained. Other cells, especially erythrocytes, take this stain, and should be identified if they are present. They may have been destroyed in the soaking process. A vigorous search should be made for the tails of the spermatozoa.

Vaginal Blenorrrhea.—Noninfectious leucorrhœa may be caused by sexual excesses, dysmenorrhœa, and many other conditions not uncommon to feminine physiology, as well as by tumors, malposition of the uterus, etc. So far as macroscopical findings are con-

cerned, this discharge may resemble true pus; microscopically it may contain polymorphonuclear leukocytes, and then a vaginitis may be diagnosticated.

Purulent Leucorrhœa.—Under this head are not usually included the nonspecific forms of vaginitis, but only those in which the gonococcus may be demonstrated. (See Searching for Germs, page 48.)

Dental Caries.—In certain chronic diseases and in fevers the saliva may become acid, and at other times an alkaline reaction may occur. A persistent acidity is usually due to lactic acid, which is formed by certain bacteria, which often results in cavity formation. Phenolphthalein solution rather than litmus should be used as an indicator.

Mercurial Ptyalism.—Salivation may be due to many causes. Mercury may be detected in the saliva according to tests described on page 91.

Noguchi's Butyric Acid Test for Syphilis.—Noguchi found that the active lipotropic bodies causing the Wassermann reaction were contained in or at least precipitated with globulin; and that the globulin fraction is increased in syphilis. To detect this increase, he has devised the butyric acid test. This may be applied to the cerebro-spinal fluid as follows:—In a very small test tube, 3 or 4 drops of this fluid are mixed with ten drops of a ten-percent butyric acid solution in physiologic salt solution. This mixture is heated over a flame and is boiled for a brief period. Two drops of a normal solution of sodium hydrate are then added quickly to the heated mixture, and the whole boiled once more for a few seconds. The cerebro-spinal fluid employed must be free from blood.

Within two hours, a white and granular precipitate should appear which will settle to the bottom of the tube. The greater the amount of protein, the more quickly the reaction will occur. Any precipitate which forms after two hours may be ignored.

Interpretation of the Butyric Acid Test.—This reaction, though valuable in the diagnosis of the syphilitic and parasymphilitic affections, may occur in any of the acute inflammatory conditions of the meninges and in tuberculous meningitis. However all of these may be readily differentiated at least from the parasymphilitic affections where acute symptoms and fever are usually absent. The butyric acid test has been found to be present in poliomyelitis. Normal cerebro-spinal fluid may give a slight turbidity but never a granular precipitate within two hours. The test is scarcely as delicate as the Wassermann in its positive phase; but the negative butyric acid test in the diagnosis of syphilitic and parasymphilitic conditions, is much more reliable than the negative Wassermann or Perutz (see page 199): the former excludes syphilis, but the latter do not. In case the Perutz test is negative, the butyric acid test should be carried out. A negative butyric acid test almost excludes the possibility of true tabes or general paralysis.

The reaction is also of value in differentiating between the various forms of acute meningitis and the other infectious diseases. Thus in typhoid, typhus, malaria and the exanthemata, the reaction is negative.

CHAPTER IX.

DIAZO VERSUS WIDAL.

This chapter is designed to set aright the puzzled practitioner, and it is probable that many of the recommendations would hardly find favor with hospitals or college clinics. Were it not for the purpose of comparison, these two investigations would be included, respectively, in *The Urine in Disease* and *Searching for Germs*.

EHRLICH'S DIAZO REACTION.

Advantages in the Bedside Diagnosis of Typhoid.—These advantages are as follows:

1. It occurs early—that is, when a final diagnosis is important. It is expected during the first week, and it is often seen from the third to the fourth day after the onset. It is commonly observed before the rash appears (Osler). The authors have noted its presence in cases not yet bedfast—that is, during that period of lassitude, headache, and chilly feeling which so often precede the onset proper—and patients with such symptoms have been sent from the consultation room to the sickbed with a fairly safe diagnosis.

2. The diazo test may be easily and rapidly completed at the bedside, requiring three solutions, a test tube, and three minutes' time.

3. The reaction is present in almost every case of typhoid.

4. The reaction is, in a practical sense, specific. It occurs in other diseases, but, with the exception of a few cases of miliary tuberculosis, these should rarely be confused with typhoid. In these cases of acute miliary tuberculosis the reaction is rarely, if ever, present during the first week.

5. The diazo reaction may differentiate between a relapse and a complication when all other signs fail, and it often reappears before or is coincident with recurrence of the fever, but is not ob-

References.—Sahli, Emerson, Simon, Wood, Boston, and other works on clinical diagnosis.

served in connection with appendicitis, perforation of the gut, or in iliac thrombosis.

Disadvantages of the Diazo.—Ehrlich's test is not without its shortcomings, as here noted:

1. It is not absolutely pathognomonic.
2. Unfortunately the diazo reaction is not always present. A negative test does not imply that the disease is not enteric fever, but the percentage of cases in which the diazo is really absent must be small indeed.

Technic of the Diazo Reaction.—The following apparatus and reagents are necessary.

Solution 1—sulphanilic acid; saturated solution in 5-percent hydrochloric acid.

Solution 2.—sodium nitrite; $\frac{1}{2}$ -percent aqueous solution.

Solution 3—aqueous ammonia.

Test tube.

Medicine dropper.

The three solutions may be obtained from reliable manufacturers. Put 51 drops of urine in a test tube and add 50 drops of solution 1 and 1 drop of solution 2. Shake thoroughly. Then add quickly about 5 drops of ammonia, allowing it to run down the side of the test tube in such a manner as to form an upper layer. If the test is positive, a deep-pink or rose-red colored ring will appear at the junction of the liquids. Shake the mixture. The entire bulk and also the foam should become red, the coloration of the foam being most characteristic of the reaction. If the urine is allowed to stand until the next visit, a dark bile-green sediment may be present, and, while of value, its absence does not indicate a negative test. The authors observed one case in which this precipitate was formed immediately on the addition of the ammonia and became very dense after one-half hour. A pink color which formed at first was not detected fifteen minutes later. The patient was delirious at the time, but eventually recovered. No satisfactory explanation of this phenomenon was ever proposed.

Pseudo-Diazo Reactions.—In health, salmon- or orange-colored rings may be observed, but not the characteristic pink or rose-red colors. The foam is not colored, and the green precipitate fails to form. Where large amounts of urine are passed, the causative factor may be so diluted that a positive reaction is obtained with difficulty, or not at all.

Salol, betanaphthol, and opium, when administered, may cause a pseudo-reaction. The foam is not, however, pink or red, but is colorless or yellow, and the green precipitate never forms. Bismuth subgallate and the tannic acid derivatives prevent the positive diazo reaction from appearing, and it is due largely to failure to take this into consideration that the total percentage of positive tests is held lower than is really just. If jaundice is present, a dark, cloudy discoloration may obscure the true reaction.

In certain cases of pneumonia, which may or may not simulate typhoid, a yellow color may appear on the addition of solution 2. Ammonia changes this to a lemon tint. Such a reaction, "egg yellow," is said to have been observed in cases of typhoid fever.

In certain affections, as erysipelas, rheumatism, and other diseases not easily confounded with true typhoid, the true diazo has been observed.

Modifications or Substitutions for Diazo Reaction.—These are listed, but not described in detail.

1. **GREENE'S MODIFICATION.** The test is said to be more delicate when double the amount of solution 1 is used. With this modification the same amounts of the other reagents are used.

2. **FRIEDENWALD'S SUGGESTION.** Paramidoacetophenon is substituted for the sulphanilic acid.

3. **EHRlich's MODIFIED DIAZO.** Where dimethylaminobenzaldehyde and other reagents are employed. (See page 114.)

4. **RUSso's REACTION.**¹ Where methylene blue is used.

Sources of Error in Technic.—These are few, but important. The instructions, if followed minutely, may be depended upon. One of the most frequent causes of difficulty may be traced to adding the ammonia layer too slowly. When this occurs, the reaction may not take place. Other sources of error may be recognized by a careful study of the pseudo-reactions.

Value and Limitations of the Diazo Test.—From what has been said, the value of this reaction may be summed up about as follows: With certain limitations, it is especially useful as a bedside diagnosis.

¹ This is a simple and seemingly valuable method of differentiating between typhoid fever and acute miliary tuberculosis. The urine of the former will be turned a beautiful emerald color when 4 drops of a 1:1,000 aqueous solution of methylene blue are added to 4 or 5 cc. of the sample. In the latter disease only a bluish or greenish tinge is obtained. It is advisable to use a normal urine in a control test if there is any question in regard to the color changes. As the positive reaction occurs in other pathological conditions, it is valuable only in the differentiation named, and should always be used in connection with the diazo.

tic procedure, and these circumscriptions have been emphasized under disadvantages, errors, pseudo-reactions, etc.

WIDAL REACTION.

Widal Reaction Defined.—While typhoid agglutination tests in general are considered, the technic described will deal only with the suspensions of dead cultures and with macroscopic observations. These seem to be not only sufficiently reliable procedures, but are specially adapted to bedside work, and the original test could hardly be made except with the equipment of the large laboratory.

Advantages of the Widal Test.—These advantages are as follows:

1. It is pathognomonic of enteric fever, thus differentiating it from acute miliary tuberculosis.
2. May occur in those few cases where the diazo test is absent, though not usually so early.
3. Its appearance, even if too late for diagnosis so far as treatment is concerned, serves to complete hospital records, death certificates, vital statistics, etc. The reader must not infer that the Widal is invariably a late reaction.

Disadvantages of the Widal Test.—These disadvantages are as follows:

1. It is of little or no prognostic value because it does not vary or reappear with a relapse, and can not therefore differentiate it from serious complications.
2. Takes too much time to perform. Even with the simple technic described, a diagnosis must be delayed for at least a few hours—a long time when we consider that we are dealing with an acute disease.
3. Although sometimes present on the fourth day, it usually appears late. Some observers (Stitt and the authors) do not recommend it as a routine procedure until the second week.
4. It is not always present in true typhoid. Although it usually appears at some stage of enteric fever, its invariable presence in this disease has yet to be proven, and a negative reaction does not imply no typhoid. So far as percentage is concerned, one thing is certain—during the early manifestations of the disease, when diagnosis from a therapeutic standpoint is most important, the

diazo reaction occurs in much higher proportion of all cases than does the agglutination test.

Technic of the Widal.—The general practitioner should appreciate the efforts of certain manufacturers in providing the simplified Widal test, but the authors do not recommend the expensive outfit. The manufacturers are usually willing to supply the dead cultures of the typhoid bacilli, properly preserved and suspended, without the fancy pipettes, etc. The remainder of the apparatus consists of two homeopathic vials.

The technic is not complex. Add about 1 dram of the suspension to each vial. One of these is corked and serves as a control. Into the other add 2 or 3 drops of the patient's blood directly from a finger prick. Shake and cork. Set aside both vials and examine in several hours. These bottles can not be carried in the pocket without interfering with the test, so that, unless the physician's office is near, a return call is necessary. Caution the family not to touch the vials. A positive test is observed when the suspension becomes granular or milky and the germs sink to the bottom, leaving an upper clear fluid. The control should remain cloudy.

Bass and Watkins' Rapid Method for Widal.—The blood is diluted by dissolving it in approximately four times its volume of water. Then one or two drops of this diluted blood are mixed on a microscopic slide or other piece of glass with an equal quantity of the suspension of dead typhoid bacilli. The slide is tilted from side to side or end to end in order to keep the mixture flowing back and forth. If the reaction is positive, a grayish-mealy sediment appears within one minute, usually much more quickly. This consists of agglutinated bacilli and is easily seen with the unaided eye. It appears in the fluid around the edges first, and tends to collect there. If the agglutination is continued, the clumps increase in size for two or three minutes. With blood giving a weak reaction, the appearance of the sediment is not so rapid as with stronger reacting blood. It is useless, however, to continue the test longer than two minutes, for, if the reaction has not occurred within that time, it will not ensue at all. When the reaction is negative, no agglutination occurs; and the mixture remains as clear and unchanged as when placed upon the slide. The suspension is best made up containing ten thou-

sand million dead typhoid bacilli per cc. in 1.7 percent sodium chloride solution to which one percent of formalin has been added. Keep well stoppered in an amber bottle and in a dark place.

Table of Comparisons.—It will be observed that this chapter has been written for the needs of the general practitioner, and the comparative table given below has been prepared with the same object in view. Typhoid is an acute and dangerous disease. Correct therapeutic measures must be instituted promptly, and delays, unless made necessary by submitting specimens to distant laboratories, must be avoided. The technic and significance of these reactions are so simple that the attending physician has only himself to blame if his patient dies “waiting for a diagnosis.”

DIAZO.	WIDAL.
1. Appears early in typhoid fever.	1. Appears in typhoid fever.
2. Reappears with relapses, but not with complications.	2. Relapses do not influence the test.
3. Technic rapidly completed.	3. Technic in simple form takes several hours.
4. Does not differentiate acute miliary tuberculosis; when delayed until the third week, suggests tuberculosis of a general type.	4. Not present in acute miliary tuberculosis.
5. Not always present.	5. Not always present.
6. Occurs in other diseases not easily confused with typhoid.	6. Occurs in no other disease.

The authors have no intention to detract from the Widal test, but simply desire to classify it properly and then to emphasize those advantages of the diazo reaction usually overlooked or neglected. The physician may make both tests, but should not omit the diazo.

Less Frequently Applied Procedures.—Both the microscopic and macroscopic tests with the living bacilli are best conducted by the larger laboratories, but, in spite of emphatic statements to the contrary, these seem to possess no special advantages over the test here described.

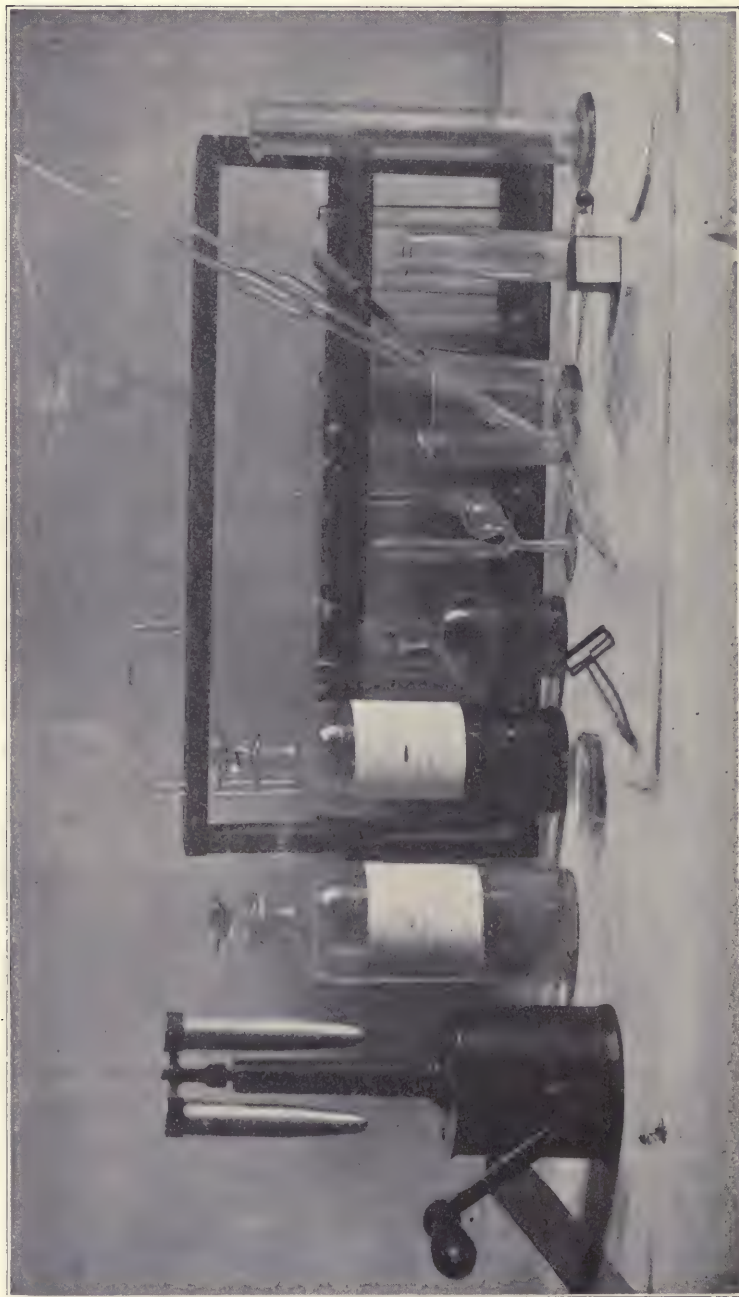


Fig. 27.—Apparatus for urinalysis.

CHAPTER X.

THE URINE IN DISEASE.

Apparatus.—Does not include that used in the urobilinogen test.

- | | |
|---|---|
| 1. Nitric acid, concentrated. | 9. Phenolphthalein, 1-percent alcoholic solution. |
| 2. Centrifuge or sedimentation glasses. | 10. Pipette, long. |
| 3. Ferric chlorid solution, strong. | 11. Silver nitrate solution, 15-percent. |
| 4. Graduate. | 12. Saccharimeter (Einhorn). |
| 5. Haines' solution. | 13. Squibb's urea apparatus. |
| 6. Lead acetate solution, strong. | 14. Urinometer. |
| 7. Medicine dropper. | 15. Window glass, 2x2 inches. |
| 8. Microscope and accessories. | |

Scope of This Chapter.—It has been the aim of the authors to select the best tests, and to describe them simply, but thoroughly, with a view to assist the physician who must do his work hurriedly and who must often search through dozens of methods to find the one which he may properly apply without laboratory luxuries and one upon which he may safely depend. In many cases a diagnosis without urinalysis is valueless, but, on the other hand, it may be stated that “conclusions drawn from the water **alone** are as brittle as the urinal containing it.”

Amount, Significance, Etc.—The normal amount varies from 30 to 45 ounces every twenty-four hours, and the normal and pathological variations bear the following indications:

Increase of Urine. Due to cold, diabetes, amyloid kidney, interstitial nephritis, some brain tumors, and following fever.

Decrease of Urine (not Including Anuria). Due to hot weather and perspiration, parenchymatous nephritis, diarrhea, hysteria, and during fevers.

Specific Gravity, Significance, and Estimation.—This varies normally between 1.012 and 1.025, being high or low respectively

References.—Memminger: *Diagnosis by the Urine*; Tyson: *Examination of the Urine*; Saxe: *Examination of the Urine*; Rieder: *Urinary Sediments*; all works on clinical diagnosis, especially Sahli, Boston, Simon, Wood, etc.

as there is little or much urine passed daily. In parenchymatous nephritis it is high, and there is little urine; in diabetes there is polyuria, but a high specific gravity; in insipid diabetes and interstitial nephritis there is a persistent polyuria, with a low specific gravity.

Estimation of the specific gravity by means of the urinometer, when carefully done, is an entirely different procedure from that usually conducted even in a perfectly arranged laboratory. A correct reading is impossible where the following precautions are overlooked:

1. All foam should be taken up from the surface of the urine by means of a piece of filter paper or blotter before the bulb is immersed.

2. The urinometer should float freely toward the center of the sample, and should not be in contact with the inner surface of the cylinder.

For practical purposes, temperature corrections are not necessary, providing the sample is neither very cold nor has been heated.

Color, Significance.—The color of normal urine varies from that of copper to brass, and the color changes bear the following indications:

Colorless urine—polyuria; red urine—fresh blood, urates; brown urine—blood, urates; smoky urine—blood; gray urine—pus, albumin; blue urine—indican, methylene blue; black urine, on standing—alkapton formed normally or after the administration of aromatic series of carbon compounds, the color usually first appearing at the top of the sample and proceeding downward.

Odor, Significance.—There is a characteristic normal odor due to certain volatile organic substances. Urotropin administration imparts to the urine an odor of formaldehyd. The sweet odor of diabetes needs no comment. A urine, standing, gives off odors characteristic of alkaline fermentation (page 121, 122).

Transparency, Significance.—A normal urine is usually clear, but may contain urates when voided, especially after exposure to cold, and may contain amorphous phosphates after a large meal. A slight cloudiness may be due to those small quantities of mucus and cells normally present.

Marked cloudiness is usually pathological to the fresh urine. If due merely to salts, a little heat and a drop of nitric acid will clear

it. Pus, albumin, and blood do not disappear during the process, and their identification must be accomplished by other tests.

Reaction, Significance, and Determination.—A slight acidity is normal. A slight alkalinity can not, in many cases, be termed abnormal. Normal acidity is not dependent on, nor perhaps influenced by, uric acid, but is caused by certain phosphates.

Marked alkalinity of freshly voided urine may suggest cystitis, but alkalinity of a decomposing urine has no clinical significance. A more complete treatment of this subject is found under the comparison of acidemia and acidosis (page 123).

The ordinary litmus test is worse than a waste of time in practical urinalysis. Acidity versus alkalinity may be first solved by the more delicate indicators, and these serve not only to draw finer distinctions, but actually give some idea as to the degree of the reaction. The authors use a dilute solution of phenolphthalein, such as is employed in the gastric analyses. In acid or neutral media this solution is colorless, but, if one drop is added to an alkaline solution, a prompt color reaction takes place, varying from a light-pink to a deep-crimson, depending on the degree of alkalinity. A simple method of estimating urinary acidity is as follows:

Add to the urine a drop of the indicator. A colorless solution signifies that the urine is either neutral or acid. Now add a drop of the sodium hydrate solution used when determining the percentage of total acid in the gastric juice (Chemistry and Biology of the Gastric Juice, page 76). If the reaction was neutral, a pink or red color will appear. The acidity may be determined approximately in degree by the number of drops of the alkaline solution necessary to impart to the sample a permanent red color, and this permanency can be tested by stirring thoroughly. In case exact readings are desired, titration may be conducted as described in Chemistry and Biology of the Gastric Juice (page 76).

Significance of Albumin.—The sources of urinary albumin are many and its etiology varied, but albumin is not expected to be found in a freshly voided normal urine. The following list will serve to give an idea of the principal sources of protein bodies:

Serum Proteins (which may or may not cloud the voided urine).
1, fevers; 2, diseases of the blood; 3, poisonous drugs; 4, accompanying heart disease; 5, certain functional and dietetic types; 6, organic kidney lesions.

Urinary Suspensions of Cellular Elements. 1, pus or epithelial cells in considerable numbers; 2, blood cells; 3, bacteria.

Choice of Tests.—The heat and nitric acid test—not the “nitric acid and heat” test—when properly conducted, often suffices, but can not be recommended as the main procedure, nor as the choice of methods when only one test is used, **as it will not demonstrate traces of albumin.** The method is, however, valuable, and should be included mainly to rule out urates, which are dissolved by the heat, and earthy phosphates, which are precipitated by a rise in temperature, but disappear on adding the acid. If an effervescence occurs on adding the acid, it is due to the formation of nitrogen gas or carbonic acid gas, depending upon the reaction of the urine.

It is, however, the trace of albumin that concerns us in diagnostics, and this small amount, as in diabetes and interstitial nephritis, may mean much more to the attending physician or life insurance examiner than any other single symptom. The first described method is merely a modification of Boston’s pipette method, a medicine dropper, such as is used by every physician, being substituted for the regular pipette.

Boston’s Pipette Method.—Compress the bulb of a clean medicine dropper (Fig. 28), immerse the point in a sample of the urine and slightly relax the pressure on the bulb, when a small quantity of the urine will enter the tube. Carefully wipe off the outer surface of the tube, immerse the point in a small quantity of concentrated nitric acid, and again relax the pressure sufficiently to draw up a column of the liquid. If albumin is present, a distinct white ring forms at the junction of the liquids. This modification offers no advantage over Boston’s method, except that of a bedside diagnosis, where a medicine dropper and a bottle of nitric acid may be conveniently used. It is more simple, and at the same time more likely to show the trace of albumin, than the heat and nitric acid method, although both tests should, if possible, be made.

Heat and Nitric Acid Test.—Fill a clean test tube half full of urine and boil, when a cloudiness may result, which may be due either to phosphates or to albumin. The addition of a few drops of nitric acid will clear the former, but not the latter. In rare instances, calcium phosphate may be dissolved with difficulty.

Filtration of the Urine.—Filtration of a cloudy urine is advisable when searching for traces of albumin, and will remove all

cells and most bacteria. In case, however, the physician is compelled to make the examination at the bedside, he may often omit it, comparing the tested sample with one which has not been examined, and in this way he will be frequently able to note marked



Fig. 28.—Modification of Boston's pipette test for albumin.

differences in the amount of albumin present. In other words, while it is advisable to use only a clear urine when testing for urinary albumin, the absence of such apparatus should not interfere with making the test, and it may be possible to obtain in the household an ordinary tin funnel and a piece of paper suitable for the

procedure. Contrary to the general idea, the laity, as a rule, do not discredit these examinations.

Estimations of Albumin.—It is indeed strange that the trace of albumin is often of greater prognostic import than the large flaky or putty-like precipitate, and for this matter the reader is referred to the larger books for the various albuminometers.

Sources of Error.—It does not necessarily follow that an albumin reaction indicates a renal lesion. If the urine has not been filtered, bacteria and the various cells may cause the positive test to occur. Pseudo-tests may be observed, and should be ruled out by the following methods when their presence is suspected:

1. *Urates.* These quickly dissolve when heated.
2. *Santal Oil, Copaiba Oil, Etc.* A few drops of alcohol added to the urine quickly dissolves these or prevents their formation.

Significance of Glucose.—Glycosuria may be transient or diabetogenous, and before the latter diagnosis can be made the occurrence of this condition must be shown to be persistent, or at least to persistently recur even when the carbohydrate food is somewhat diminished. The presence of diacetic acid or of the other acetone bodies signifies sugar diabetes. In case of doubt, a record of twenty-four-hour amounts and specific gravity tests may clear up the diagnosis. Glycosuria is merely a sequence of hyperglycemia.

Detection of Glucose.—The authors recommend Haines' test, not merely because it is simple, but because it gives such satisfactory results. A small bottle of the reagent may be obtained at any pharmacy, and does not necessitate a fresh preparation for each test. In time, however, a reddish precipitate will settle to the bottom of the bottle, when a fresh supply should be obtained. On boiling this reagent, before the urine is added, precipitation should not occur. If albumin is present in the sample to be tested, it should be removed previous to the examination. The procedure is as follows:

1. Boil in a clean test tube for two minutes 1 dram or more of Haines' reagent.
2. Hold in the light to make certain that no change has occurred.
3. **Quickly** add from 2 to 7 drops, **not more**, of the urine.
4. Keep the reagent boiling before the urine is added, but do not heat after this has been done.
5. A **heavy** yellow, brown, or red precipitate indicates the presence of glucose. A slight flaky white or dirty collection of crys-

tals indicate merely a partial reduction of the reagent, and do not indicate that glucose is present. The typical precipitate is heavy, both in appearance and in reality, settling to the bottom of the test tube like sand, and, when once seen, is never confused with the pseudo-reactions.

Estimation of Glucose.—This may be necessary in certain dietetic tests, but certainly never as a prognostic procedure, the increase of the acetone bodies serving the latter purpose.

The Einhorn saccharimeter offers a method that is simple and fairly accurate. Its chief objection is that a wait of twenty-four hours or longer is required for its completion, a criticism that seems inconsequential when the chronic nature of diabetes is considered.

Sources of Error.—Haines' test is satisfactory when directions are properly followed. Glucosides and certain volatile oils, when taken internally, are often excreted by the kidneys. When boiled, they break up into simple substances, including either free glucose or similar bodies, which would, of course, reduce Haines' solution; but these will not, however, cause any trouble if the contents of the tube are not heated after the urine is added. As noted above, albumin will interfere with the test, and should, if possible, be precipitated and filtered before testing for glucose. The physician may advantageously at some idle moment attempt this test with a sample of urine to which a little glucose has been added. A qualitative fermentation test, with a little yeast in an ordinary bottle, leaves no doubt as to the presence of glucose.

Significance and Detection of Bile.—Biliary products, such as are finally excreted by the kidneys, appear in the urine as urobilin, and are the cause of its normal yellow or orange tint. In jaundice, however, bilirubin and its oxygenated derivatives may be present in the urine. The best test for these may be made by merely shaking well the sample and observing carefully the color of the foam. In normal urine this is white, but, if abnormal biliary pigments are present, a yellow, green, or brown coloration will be noted. For practical purposes no other test need be attempted.

Significance of Blood.—Fresh or changed blood may come from any portion of the urinary apparatus. It is claimed that renal and vesicle hematuria may be distinguished by several mechanical methods. One of these requires the urine to stand twenty-four hours in a conical glass. In vesicle hematuria all blood will have

settled to the bottom of the glass, but in the renal forms the entire liquid will remain smoky. Fresh blood indicates, most probably, a vesicle source. Casts containing blood cells point to renal origin.

A red urine may be due to fresh blood or to urates. Heating the sample causes rapid solution of the latter, but has no effect on the former.

The Urobilinogen Test for Hepatic Function.—Neubauer and others have shown that the Ehrlich's modified diazo reaction (see page 102) is not after all a test for typhoid fever, but often occurs here and elsewhere inasmuch as the liver when involved sometimes permits urobilinogen to escape unchanged into the urine; and it is this substance which gives the test. It would thus seem that we have found a very valuable as well as exceedingly simple method of determining whether or not the "liver function" is normal; and this may be of considerable diagnostic profit. Thus, for example in the cirrheses, where most of the cells of the liver are simultaneously involved, this organ may be unable to "work up" the urobilinogen. In other words, so many cells are injured that the remainder are unable (by virtue of their inherent possibilities to undergo compensatory hypertrophy or other change) to complete the work expected of that organ. The test appears to be a very sensitive one, but never occurs in perfect health. It appears to be of great value in the diagnosis of those liver ailments where the greater part of the organ is affected; viz., passive congestion, cirrhosis, biliary obstruction, severe fevers with involvement of this organ, hepatic syphilis and angiocholitis. Minor hepatic troubles affecting most of the cells may give rise to urobilinogenuria whereas severe diseases of that organ affecting only a small portion of it may cause no such condition because enough cells are left uninjured to assume the whole work laid out for that cell community.

Do not heat the urine or the reagent, as the results will be confusing. Two drops of the testing solution are added to one dram of the urine. The mixture is shaken and set aside at room temperature. If urobilinogen is present, a beautiful cherry-red color will develop after a time varying from a minute to a couple of hours. A yellowish or pinkish tint cannot be regarded as positive. The testing reagent may be made up and kept indefinitely. It is prepared by dissolving one gram of paradimethy-

minobenzaldehyde in 10 cc. of pure hydrochloric acid and then adding five drops of alcohol and enough distilled water to make up 50 cc. total quantity.

Diazo Reaction.—(See Diazo Versus Widal, page 101.)

Significance of Urea Content.—Normally, about 500 grains of urea are passed daily, but this output varies under physiological conditions. It is only when the urea runs very low that a pathological significance may be applied. In certain forms of Bright's disease, and in pregnancy where eclampsia is feared, its estimation may be attempted mainly as a prognostic procedure.

Estimation of Urea.—Any method requiring the making up of fresh quantitative solutions for each estimation finds small favor with the practitioner. The Squibb apparatus requires but little of this work, avoiding at the same time the use of the irritating bromin. This apparatus is sold by instrument dealers, and is accompanied by full directions for using.

Significance of Uric Acid.—In small amount, uric acid is normal, but in increased quantities—due to unknown causes—it may lead to the formation of urinary calculi, to gout, and to other forms of arthritis.

Detection of Uric Acid.—In a cold urine, uric acid and its homologues appear as a brick-red precipitate. A little heat will cause their solution, thus differentiating them from blood.¹ A simple test for one of the compounds accompanying the uric acid proper may be made by adding 1 or 2 drops of a sugar of lead solution to the sample, when a flesh-colored precipitate will be formed. A microscopical examination may reveal in the cold urine characteristic uric acid crystals.

Estimation of Uric Acid.—That true lithemia occurs, and that an increase of uric acid in the secretion of the kidney proves its existence, are not unquestioned medical truths. This statement does not refer to true gout, but to the many ailments usually attributed to this normal urinary salt, and for these reasons the estimation of uric acid can not properly be considered a common procedure, nor has it earned a place in the physician's laboratory.

Significance of Chlorides.—These are normally present in the urine. Continued fever or serious kidney lesions cause decrease of chlorides. Cessation of excretion of chlorides in urine forms

¹ Only the urates are dissolved, and such quantity of the acid itself as may be present is so small that the test loses none of its value.

the basis of an unfavorable prognosis in pneumonia. After the crisis is successfully passed, the chlorides are increased in amount.

Estimation of Chlorides.—The following plan is simple, and at the same time very useful;

1. Remove, if necessary, any albumin that may be present.
2. Filter if necessary.
3. Take 2 ounces.
4. Add 1 drop of nitric acid.
5. Add 3 drops of a 15-percent solution of silver nitrate.

A normal amount of chlorides produces thick, curdy masses, whereas greatly diminished chlorides cause only a slight cloudiness. More delicate estimations are unnecessary for prognostic purposes.

Significance and Detection of Diacetic Acid.—Diabetic coma is due to betaoxybutyric acid, but, as the presence of this is detected with difficulty, search is usually made for diacetic acid, a body closely related and usually formed simultaneously. Diacetic acid has been found in other conditions, and is of little diagnostic import, but its appearance late in sugar diabetes is of great interest in that a coma is likely to follow, and hence its identification becomes of great prognostic interest. The sample must be fresh when tested, as this acid is very volatile. The urine must be added to the ferric chlorid, else filtration will be necessary in order to see the color reaction. The test is as follows:

1. Into a test tube pour about 2 drams of a clear, strong solution of ferric chlorid.

2. Add 1 or 2 drops of the urine. A wine-red color indicates the presence of diacetic acid. The reagent should not be added to the urine, as is generally advised. If compounds of the aromatic series are being exhibited, these will give a pseudotest. In such contingency, recall that diacetic acid is partially decomposed by boiling the urine just prior to the test, thus reducing the intensity of the reaction.

Collecting the Sediment.—Whether gravity or the centrifuge is employed to separate the sediment from the liquid is immaterial, except perhaps that the first method requires too much time. The sediment may be collected from beneath the supernatant liquid by the following method:

1. Use a long pipette. If a medicine dropper is employed, the technic may be modified to apply to its use. It is sometimes advisable to select this sediment from the bottom of a bottle, and

therefore a small glass tube drawn to a point should be a part of every physician's equipment. With the index finger pressed against the top, immerse this pipette so that the point comes in contact with the sediment.

2. Now carefully relax the pressure on the upper end, and some of the sediment will be drawn into the tube. Quickly apply the pressure and remove the pipette from the sample. In case the sediment appears to be in strata, specimens from the several layers may be taken.

3. On a piece of window glass (2 x 2 inches) drop at different places some of the sediment, and examine with a low-power objective and narrowed diaphragm. In case there is any question concerning the recognition of certain elements, drop on a cover glass, slightly open the diaphragm, and use a higher magnification.

Pus.—Separate chemical and microscopical considerations of pyuria are almost impossible. When its origin is in the kidney tissue, only a few cells are usually found, and the companionship of certain other elements—as casts, crystals, and epithelial cells—may aid in determining the source. A renal pus shows a tendency to appear and disappear at intervals, is acid, shows no tendency to thread formation, and its cells are usually well preserved.

In cystitis, if acid, differentiation may be impossible, but if alkaline, as is usually the case, the cells tend to be swollen or destroyed.

In urethritis the first urine voided should contain much pus, while that collected in a second glass exhibits but little. It shows a tendency to thread formation—clap threads.

The pus cell is usually a polymorphonuclear leukocyte, but lymphocytes may be present. There should be little difficulty in ruling out epithelial cells and unnucleated red corpuscles, and in case of doubt the nuclei may be brought out by adding to the drop of sediment a drop of 1-percent acetic acid. The pus of pyelitis is differentiated with difficulty. When desirable, smears and stains may be made for the tubercle bacillus, but the search is too often very discouraging.

Casts—Etiology, Types, and Differentiation.—A complete classification of these elements is left to the larger books. For practical purposes, it is not necessary to draw fine distinctions—any type may be expected in the several kidney lesions, although certain forms may be more typical of an interstitial inflammation than of a parenchymatous nephritis.

The recognition of tube casts should not be a difficult matter, but nevertheless much confusion exists in regard to this point among practitioners. There is no doubt, however, that one who has once really seen and identified these elements is never perplexed in the future. Some of the more common sources of error are the following:

1. Mistakes in identity. The harmless epithelial cell and the extraneous cotton fiber are only too often mistaken for casts.

2. Working with too much light. Many persons neglect to properly narrow the diaphragm in making examinations of the unstained preparations of urinary sediment, as well as of other smears and sections which have not been treated with a dye.

3. Attributing to the flexible and mucus-like cylindroids undeserved individuality.

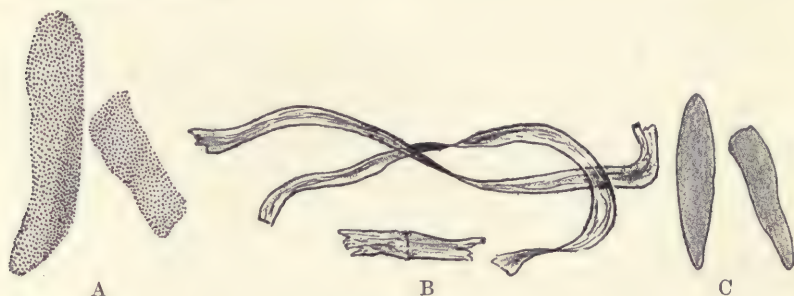


Fig. 29.—Artifacts. A, granular casts; B, vegetable fibers; C, epithelial cells which have lost their nuclei.

The cast is formed in the uriniferous tubule, and the latter may, in fact, be considered its mold; so that, if there is blood in these tubules, there will be a blood cast; if there is pus, a purulent cylinder will result, etc. The granular casts represent, in all probability, necrotic epithelial cells which line the uriniferous tubules; in fact, certain of these casts contain large remnants of these cells or the cells themselves. There may be other casts representing degenerative or necrotic changes, as certain hyalin or fatty casts. When large numbers of bacteria are included, a so-called bacterial cast may result.

In certain pathological conditions individual types may predominate, although any or all of the others may be present. Thus, in an acute nephritis, search is naturally made for the epithelial, blood, and leukocyte casts, while the granular and hyalin forms

are more characteristic of chronic Bright's disease. Epithelial casts are rarely found in simple interstitial types of kidney disease. Bacterial casts indicate a very grave prognosis. In diabetes there are usually expected to be found, at some stage, short hyalin casts.

A cast should not be confounded with a cotton or flaxen fiber, as there are few or no points of resemblance, which is clearly shown in Fig. 29. Often, though not always, these fibers lie on the cover slip, and the objective must be lowered before any of the urinary sediment comes into view. An epithelial cell in which the nucleus shows but faintly or not at all, and the edges of which tend to "roll," seems often to confuse the microscopist initiate. In case of doubt, a little acetic acid or a drop of slightly acidulated



Fig. 30.—Some typical epithelial cells from the urinary passages. A, squamous cell of vagina and urethra; B, caudate cells from pelvis of kidney, ureter, and bladder; C, cylindrical cell from the upper portion of the male urethra; D, polynuclear cell, same origin as tailed cell; E, two renal cells.

methyl green may demonstrate a nucleus. **Do not forget to narrow the diaphragm**, which admonition is emphasized because failure to do this is one of the chief reasons why most practitioners fail to see casts. A real cast is, however, rarely mistaken for an epithelial cell or a bit of extraneous matter, as the outlines are too clearly cut and the morphology is too characteristic. It is therefore a fairly safe conclusion that where doubt exists the element in question does not properly belong to the pathologic nomenclature. Casts are demonstrated with difficulty in a urine voided several hours before.

Blood Cells.—Blood is identified microscopically by the presence of red cells. These, unless hemorrhage is recent, are rarely intact, but are swollen, and usually lack some of their pigment, at times even forming blood shadows (Fig. 30).¹ Casts signify renal

¹ It follows that in some urinary specimens, especially those where the examination has been delayed, the erythrocytes can not be identified by the microscope and the presence of hemoglobin must be demonstrated. For this purpose Meyer has devised

hemorrhages. Here, as with pus, the coexistence of certain other elements may indicate the location of the bleeding point.

Epithelial Cells.—A few desquamated epithelial cells may be found in a sample of normal urine, and large numbers of these elements point to pathological processes. Unfortunately the shape of the cell rarely aids in diagnostics, as the various types are so widely distributed. Ordinarily the characteristic cell of the vagina or urethra is referred to as a flat cell (Fig. 30). Vesicular denudations, as well as those from the ureter and kidney pelvis, usually include characteristic star-shaped and "tailed" cells. Very small round cells, with spherical nuclei, are most likely to come from the uriniferous tubules.

Chemical Sediments.—These are of less diagnostic significance than the organic elements. The following is a brief classification of the more common crystals:

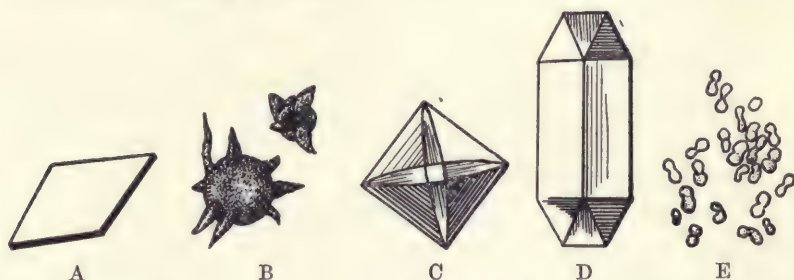


Fig. 31.—Comparison of the more usual forms of the common crystals met in urinary sediments. A, uric acid; B, ammonium urate; C, calcium oxalate; D, triple phosphate; E, calcium carbonate.

1. *Uric Acid and Urates (Brown Color).* (Fig 31.) Soluble only in warm solutions. Typical uric acid crystals are of a rhomboid shape, and those of ammonium urate have an appearance not unlike that of the common cockle-bur. There are many variations from these characteristic forms, but certain points of resemblance are found in the less frequently observed types. Occur in normal urine, but are increased in gout.

2. *Calcium Oxalate.* Show typical envelope shapes. Soluble in hydrochloric acid and insoluble in acetic acid. Of little patho-

an excellent test. Prepare the reagent by heating 4 grams of pure phenolphthalein, 20 grams of potassium hydrate, about 15 grains of zinc dust, and 200 cc. of distilled water. Prolong the heating until all the pink color disappears, and then filter and bottle. Having obtained a good solution, which may be kept a long time, the test itself is simple. Add a few drops of this reagent and a drop of peroxid to a few cubic centimeters of the urine. A pink coloration indicates the presence of hemoglobin. Make a control test with a normal urine.

logical significance. When present in large amounts, these crystals may irritate the ureters and give rise to symptoms similar to those noted in renal calculus. Such a condition has been termed painful oxaluria—oxaluria dolorosa.

3. *Triple Phosphate*. Heat causes precipitation rather than the solution noted with urates. The coffin-lid shape is typical. Occurs in ammoniacal fermentation, whether in a diseased bladder or in the urinal.

4. *Calcium Carbonate*. Occurs as small transparent spheres, which give off gas (carbon dioxide) if acid is added. Occur under the same conditions as the triple phosphate.

Bacteria in General.—A few bacterial cells may be present in a normal urine freshly voided into a sterile receptacle. Urine, on standing, soon becomes contaminated by microorganisms of the air, and “spoiling” occurs. In a majority of cases this fermentation is alkaline in character (ammoniacal), and such a urine abounds in germs of many varieties.

Parasites.—The tubercle bacillus may, with difficulty, be identified in cases of tuberculosis of the genital tract. See Searching for Germs (page 48) for the detection of the gonococcus. The microorganisms of many system affections, as typhoid, may occur in the urine, but are rarely considered from a diagnostic standpoint. The colon bacillus is usually blamed for an acid cystitis, whereas a bladder affection alkaline in character is often due to the bacillus proteus vulgaris. Multitudinous varieties of bacteria abound in ammoniacal fermentation of the urinal. Smegma bacilli, yeasts, molds, amebæ, and the eggs and bodies of certain animal parasites have been identified, but, with the exception of the first of these, are very rare. It follows, therefore, that a diagnosis of colon cystitis on the appearance of rods in a sample urine is as impractical as the diagnosis of consumption on the presence of a cough. Strange to say, many of these impractical procedures are often conducted by men who acknowledge that they are unable to identify a urinary cast or differentiate between albumin and urates.

Extraneous Matter.—The various vegetable fibers and starches which may be found in any microscopical preparation have been previously considered (page 30), and should be easily recognized. They are introduced accidentally as contaminations from the hands, towel, or other sources not easily determined.

Ammoniacal Fermentation.—While reference has been made to

this condition (page 108), a few special points may be emphasized. A "spoiled" urine may be found either in a cystitis or in a stinking urinal. In the former case it is most likely to be due to the bacillus proteus vulgaris, but when voided may be caused by many types of microorganisms. Under the alkaline cystitis (page 122) the findings of this condition have been listed, and they differ very little from those here noted as the fermentation in the urinal:

1. Pungent odor, due not only to ammonia gas, but to certain other decomposition products.

2. Marked alkaline reaction.

3. Presence of many bacteria.

4. Dirty white or gray sediment, which shows under the microscope the coffin-lid crystals of the triple phosphates and the spherical carbonates.

Urine of Specific Urethritis.—Contains clap threads, pus cells containing gonococci, and rarely blood.

Urine of Acid Cystitis.—Acid reaction. Slight fecal odor. Contains pus, red blood cells, and epithelium. Caused commonly by the colon bacillus.

Urine of Alkaline Cystitis.—Marked alkaline reaction. Foul odor of ammonia and putrefactive gases. Contains triple phosphates, pus, epithelium, and rarely blood. Caused commonly by bacillus proteus vulgaris.

Urine of Foudroyant Cystitis.—Characterized by much blood and pus. Caused by streptococci and other virulent germs.

Urine of Pyelitis.—Rarely shows any features different from those of acid cystitis.

Urine of Renal Calculus.—Acid reaction. Contains blood and often bits of urate or oxalate gravels.¹

Urine of Vesical Calculus.—This presents a picture of a severe cystitis, with blood and possibly phosphatic concretions.

Urine of Renal Hyperemia.—Small amounts of albumin, but few or no casts, with little or no blood. In the passive hyperemia of mitral insufficiency the urine is acid, contains many urates, and has a high specific gravity.

Urine of Uremia.—Many casts of all types, much albumin, and decreased urea.

Urine of Parenchymatous Nephritis.—Small amount of urine,

¹ The presence of oxalate crystals in the urine is not sufficient proof that a calculus is lodged in the renal pelvis or ureter. That these crystals may cause distressing symptoms resembling those of renal calculus appears to be unquestioned.

containing much blood and albumin, and of a high specific gravity. Contains, besides free blood, blood casts and many epithelial casts.

Urine of Interstitial Nephritis.—This is the chronic Bright's disease. It is characterized by much urine, containing no blood and only traces of albumin, and shows a low specific gravity. Hyalin and granular casts are usually present, but the others are rarely found. Cellular elements are generally few in number.

Urine of Diabetes Mellitus.—Much urine, usually of a high specific gravity. Smells sweet and has but little color. Glucose and the acetone bodies are present. Traces of albumin and the short hyalin casts are not uncommon.

Urine of Diabetes Insipidus.—Much urine of a low specific gravity, but shows no sugar, acetone bodies, albumin, etc.

Urine of Autointoxication.—Variable but usually small amount of urine of a high acidity, sometimes showing traces of albumin and hyalin casts but invariably contains large quantities of indican or indolacetic acid. Tests for these as well as a bedside method of estimating the acidity, may be found in the appendix. Some authorities term this condition acidemia or copremia.

Acidosis Versus Acidemia.—Harrower and other writers have pointed out these differences. A brief classification of all that pertains to the acids of the urine is here given:

1. *Normal Acidity.* To certain phosphates is due the normal urinary acidity.

2. *Uric Acid Diathesis.* It has yet to be proven that excess of uric acid in the urine increases the acidity of that excretion.

3. *Acidosis.* When the urine of sugar diabetes contains the acetone bodies.

4. *Acidemia.* A condition observed in autointoxication, and undoubtedly caused by certain acids containing sulphur, by indolacetic acid, and by other products formed in abnormal intestinal putrefaction.

Tuberculosis of the Urinary Tract.—The tubercle bacillus is not easily found, which is especially the case if much blood is present, and it is well to centrifuge before making the spreads. The smegma bacillus, though acid fast, shows no tendency to occur in clumps. If any doubt exists, it may be advisable to wash the stained preparation in an absolute alcoholic solution of 1-percent

hydrochloric acid for ten minutes, which will decolorize the smegma bacillus, but the tubercle germs will remain brightly stained.

Iodin Tests in Malingerers.—Under certain conditions a physician may desire to know whether his patient is taking the medicine as prescribed or dispensed. By adding a few grains of potassium iodid and collecting a sample of the urine, that drug may be easily detected as follows: To the urine add a little starch solution, and then add 1 or 2 drops of chlorin water. A blue color indicates the presence of the iodid.

Tampering with a Urine.—The authors have observed several instances of this kind. A quack doctor taught his patients to test for albumin and sugar, and then prescribed at intervals the oil of copaiba. One woman, insistent that she had diabetes, was finally informed that her urine contained large quantities of cane sugar. The addition of glucose to a voided urine is quite common, and Osler records one case where it was introduced into the bladder. Brick dust or iron rust may be added to give the appearance of urates or blood, and ammonia may be added, which does not, however, result in the formation of the typical coffin-lid crystals of triple phosphate, but in certain star-like crystals. A candidate for a life insurance policy may bring a bottle of normal urine to the office, whereas a freshly voided sample from the same person would show sugar, albumin, or casts. The physician must be on the lookout for deception.

Less Frequently Applied Procedures.—Some tests have no diagnostic or prognostic worth, and others of great value are rarely or never attempted by any save experts. Both of these classes have been combined and listed as follows:

1. Temperature corrections for specific gravity.
2. Detection and estimation of certain salts and other constituents of the urine.
3. Several tests for albumin and its quantitative estimation.
4. Certain bacteriological examinations, etc.

Value and Limitation of a Practical Urinalysis.—The value of the tests described depends on their proper selection and application, and, while their importance is too often underestimated, the deductions drawn from this source alone are of little value. The urinalysis is only another link in that chain by which a safe diagnosis is determined.

CHAPTER XI.

MILK AND ITS HOME MODIFICATIONS.

Apparatus.—

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| 1. Hydrochloric acid, dilute. | 7. Centrifuge and special tube. |
| 2. Sulphuric acid, commercial, slightly diluted. | 8. Microscope and accessories. |
| 3. Sulphuric acid, C. P., concentrated. | 9. Phenolphthalein, 1-percent alcoholic solution. |
| 4. Methyl alcohol. | 10. Pipette or medicine dropper. |
| 5. Fusel oil. | 11. Tincture turmeric. |
| 6. Ammonia water. | 12. Urinometer. |

The methods given here are those which are used successfully by the leading pediatricists of the country. So far as practical purposes are concerned, the formulas for the home modifications are ideal in that the mixing may be done by the housewife. These tests and estimations are exceedingly simple, but the lack of proper references renders it necessary to include in this chapter more details than might be desired. Moffitt, in the *Journal of the American Medical Association*, has ventured to criticise the absolute accuracy of the formulas, but his observations are at variance with those of the authors, who have noted that control analyses of samples after modification seem to be of surprising accuracy. It is the opinion of many authorities that it is unnecessary for these to be absolutely accurate. For the benefit of those not so well informed on the subject the authors have deemed it advisable to enter into detail.

Substitutes for Mother's Milk.—The summer months is the time when the health of the baby is generally affected, and when its food must be changed it may occur to the physician that the little patient needs modified milk, but he makes no effort to put his opinion into practice. The home modification of milk and the principles of infant feeding are so little understood by the profession that any light thrown on the subject must be of more than passing interest. Observations in a number of cases will convince

the physician that there is a science in feeding the baby which is denied the maternal milk.

The earliest work in this matter was done by T. M. Rotch and G. E. Gordon. The work of the Walker-Gordon laboratories is to be commended, but hardly comes within the scope of this book, as they are rarely accessible to the general practitioner. They furnish the milk direct on prescription, acting in the same capacity as the pharmacist, and merely see that the patient gets exactly what is ordered. A physician who is ignorant of the principles of infant feeding can place no blame on these laboratories for poor results. The absence of these facilities should not, however, render helpless the man outside of the larger cities, as an energetic physician, an intelligent mother, and a conscientious milkman can accomplish just as good results as the best equipped metropolitan laboratory.

Maternal nursing is most to be desired. The woman who refuses to nurse her child deserves censure, but the physician is often an unsuccessful social reformer. Not only is the mother's milk more acceptable to the infant, but nursing the child aids the uterus to contract and involute properly, and especially among the poor and ignorant should maternal nursing be encouraged. Severe post-partum hemorrhage, acute infections, tuberculosis, and certain chronic nervous diseases, as epilepsy, are contraindications. Maternal syphilis, where the child seems to be healthy, should be considered a contraindication, although the healthy parent may nurse with safety her syphilitic babe.

The best substitute for the milk of the mother is that of some other woman, but in America there are no slaves or peasants, and a healthy wet nurse is either a luxury or an impossibility. Hidden disease is always a menace to both parties. Mixed feedings may be deemed advisable where the milk of the mother is good, but small in amount.

Of all artificial foods, modified milk is accepted by scientific men as ideal. Its real merits are borne out by clinical experience, and the results of its therapeutic efficacy may be seen in a hospital ward furnished with scientifically prepared modified milk from the adjoining milk laboratory. The baby's organs of nutrition, however, form a machine doubtless more complex than that of the adult, and no physician should become so infatuated with this method of feeding that he would see in it perfection, as there is still much to learn about feeding the baby. Because of disappoint-

ments to those who were at first too optimistic and the ignorance of the profession as to the simplicity of the home modification, the various proprietary infant food concerns exist. The products of these concerns may have done some good, but unfavorable results do occur from their use, and experience shows that these unfavorable results are the rule. In the first place, they are stock prescriptions, and in their preparation the condition of the patient is not and can not be taken into consideration. Any intelligent physician will regard this "panacea" from about the same viewpoint that he would look on any other cure-all. A beautiful booklet may give directions how to mix it with cow's milk, but only the latter is needed, as it is cheaper, safer, and better for all babies, sick or well, and its real worth is shown by the attempts to imitate it. These statements are not made in an unfriendly spirit, but certain comparisons are presented for the guidance of the general practitioner.

Proprietary Foods Versus Modified Milk.—There have been cases in which the proprietary foods have been prescribed where they either fortunately suited the condition or were selected by a physician who understood the principles of feeding and knew the composition of the food, and who applied this knowledge to the particular infant. Several brands may have been cast aside as worthless in an exhaustive experiment, and one found to fit the case just in time to avoid fatal results. The picture of the healthy infant is shown in the advertisements, but the others who developed rickets, vomited up their food, or died are not portrayed.

The following are the advantages and disadvantages of the proprietary foods, as well as those of cow's milk scientifically modified:

Advantages of Proprietary Foods.

1. Convenient to use.
2. Suit some babies under some conditions.
3. Fairly good substitutes, when properly modified, for cow's milk **when the latter can not be obtained in a pure state.**

Disadvantages of Proprietary Foods.

1. Expensive, and usually unnecessarily so.
2. Not ideal substitutes from a scientific standpoint, as the proteins, fats, and carbohydrates are not in correct proportion even for the healthy baby.
3. Not ideal substitutes from a clinical standpoint. Unless the

food happens to suit the case, there results scorbutus, rachitis, marasmus, or even acute inanition.

4. They present an attempt to fit the patient to the cure.

5. The proteids and sugars of the cereal foods are entirely different from those of the animal milks, and, although their amounts may be modified, an alteration of their composition is impossible. Because "proteids are proteids" is no reason why bad proteids should be substituted for good proteids. All sugars are sweet, but the diabetic may safely take levulose when glucose may be poisonous to him. Sugar of milk is absent in many of these foods.

6. Menace infantile life because they are offered to the laity, and the helpless mite is intrusted to the untrained. They are prescribed according to bottle directions, and the parents, having the physician's recommendation, are under the impression that science can do no more than they in saving the baby.

7. Menace infantile life because in many cases the manufacturers presume on the ignorance or indifference of many of the profession, so that the real dangers are hidden.

8. Given straight or modified, no artificial food contains those milk enzymes known to be so necessary to the infant, unless it also contains that which is absolutely necessary—the **fresh milk of some mammal.**

Advantages of Modified Cow's Milk.

1. Approaches nearest to mother's milk, and may be further modified to suit the infant when even mother's milk, if obtainable, would fail.

Disadvantages of Modified Cow's Milk.

1. Widespread belief that the process is very difficult.

2. Ignorance of the profession not only of the process of modification, but of the principles of infant feeding.

3. Lack of laboratory facilities.

4. Indifference even on the part of recent graduates, who should know how to feed babies.

Working in the Dark.—Cow's milk is crudely modified by some physicians by decreasing all the ingredients simultaneously by diluting the milk *in toto*; then, if it is desired to bring up the fat content, cream is added, or, if the sugar is to be increased, saccharum lactose may be added. It is seldom necessary to increase the proteids, as there are enough or too much in cow's milk. At best the system is one of working in the dark, as cow's milk

varies greatly. The reason that even rickets may result may be seen in the following table, in which the proteids of the cow's milk, by dilution with equal parts of water, have been brought down to coincide with those of woman's milk, but there is a great variation in the fats.

	Proteids.	Fats.	Carbohydrates.
Cow's milk	3 percent.	4 percent.	5 percent.
Cow's milk diluted . . .	1.5 percent.	2 percent.	2.5 percent.
Woman's milk	1 percent.	4 percent.	7 percent.

There must, of course, be a good milk supply, and to assure this a testing of milk for dilution, cleanliness, etc., appears necessary, though a conscientious milkman may materially aid the physician in this matter. The family of the young patient can also aid in perfecting a suitable modified milk, but the physician should thoroughly understand the principles of infant feeding.

The Laboratory.—This is a secondary matter, but important. Each physician may have his own work-room, fitting it up for less than he pays for paregoric and infants' anodynes; or several physicians may have a laboratory in common. The worker needs a bottle of sulphuric acid and a few other reagents, and, if he possesses a centrifuge, he may obtain a fat testing tube at small expense. For convenience of description, we shall consider the laboratory work and the prescribing as being done by two different men. The actual mixing of the baby's milk is done every day by the family according to directions from the laboratory man, who is in turn instructed by the physician in charge of the case.

Writing the Prescription.—The physician may be his own analyst, but, in case another man does the work, his relations to the latter are as follows:

1. He should devise a prescription to suit the case, and, if he is not sure as to the one indicated, should study up the subject.
2. He should see that a sample (one quart) of the milk reaches the analyst. In many cases the milk dealer should not be aware, for baby's sake, that this examination is to be made.
3. The prescription should state the amount of milk required for the infant every twenty-four hours.

At this point the responsibility of the prescriber becomes that of the laboratory worker, but it is advisable to enlarge on these three points before we study the work of the milk laboratory.

Let us first consider the devising and writing of the prescription. Many of the poor results from using modified milk are due to ignorance in this matter. A physician should not merely write

R Modified Milk $\frac{3}{4}$ v
Sig., etc.

As has been stated, a knowledge of the principles of infant feeding is necessary, and, if this is lacking, it may be obtained in a short review of a book on pediatrics. The authors do not wish to overstep the scope of this volume, but emphasizing a few principles will not be out of place.

Some Principles in Baby Feeding.—There are two numbers which every physician should bear in mind—viz., 453 and 471. Although the makeup of woman's and cow's milks vary, for practical purposes the following arrangement will answer:

	Fats.	Carbohydrates.	Proteids.
Cow's milk	4	5	3 (453)
Woman's milk	4	7	1 (471)

First let us consider the healthy baby where maternal milk can not be obtained. Many of the bad effects of unmodified cow's milk in the healthy infant is due to the high proteid content of that secretion. The carbohydrates, although a lesser factor in these considerations, should also be changed in order that mother's milk may be approximated as nearly as possible. For that reason a 471 milk may be tried, but experience shows that when beginning a modified feeding it is often best to reduce also the fats, and a popular trial mixture has resulted in the 261 combination. If the latter does not agree with the baby, it may be modified, and symptoms should be the guide for modifications. Gastric symptoms indicate fat indigestion, and this substance must be increased or lowered accordingly—generally lowered. When intestinal symptoms predominate—either diarrhea or costiveness—the proteids are at fault. Early symptoms of rickets indicate that the fats must be increased at once. Such symptoms as sweating of the head, restlessness at night, constipation, beading of the ribs, and craniotables are important, but easily overlooked. Stool analyses rarely hold that value observed where the infant is sick, and the apparently pathological stools which may be passed by the healthy infant are surprising.

For the sick baby we must use those principles indicated in the text books, and we must not forget our drugs—castor oil and peppermint water. Contrasted with the healthy infant, the appearance of the stools should be noted, as this is of great importance. (See *Every-Day Stool Tests*, page 154.) An entire change of the prescription or its abolition may be necessary.

When the baby becomes older, or even with the sick young baby, it may be advisable to add certain substances found neither in the milk of the cow nor that of the human, but which clinical experience has demonstrated to be useful should certain indications arise. These have but little to do with this subject, and are really not foods, but tend toward drug medication. They have been used in the city laboratories for the convenience of physicians, and under this head come the syrup of lime, peptonization, barley water, whey, and buttermilk feedings. They should be used under the direction of the physician in charge, but, if dispensed, should not, like all other prescriptions, be refilled without his sanction.

Sample for Analysis.—This investigation is usually necessary for many reasons. In the first place, all milks differ in fat content. The variations in the other constituents are less marked, and usually need not be considered. The amount of fat in a given milk varies according to the breed of the cows, the time of the year, grazing conditions, first milk or top milk, whether the milk comes from a single cow or a herd, etc. Even if the composition of all milks were constant, the ignorance of many dealers renders it advisable to always be assured of their purity. Dr. Woods Hutchinson says that one teaspoonful of milk may contain more inhabitants than the city of New York. If these were all lactic acid bacilli, no harm would result, but there is no natural selection. Dr. Hutchinson states that 90 percent of the injurious effects of milk is due to the germs of plain, common dirt, and this comes not only from the barnyard, but from unwashed milk cans and other avoidable sources. Asepsis is no less important here than elsewhere, and sterilization, except for long journeys, is rarely necessary and even harmful in many cases.

Tampering with Baby's Food.—We are too often shocked to learn that the apparently honest milkman is disposed to tamper with his milk, and evidently infanticide is justifiable in his eyes. He will add water to increase his profits, but, worse, he will add some poison to inhibit the lactic acid bacillus, unmindful of the

other varieties which nevertheless proceed to multiply. He removes the danger sign—the milk does not “sour,” and it is impossible to determine whether it is fresh or spoiled. The milkman is saved the expense and trouble of ice, but the consumer is subject to his poisons. Analysis of the milk guards against these dangers, but it is advisable to select a milk with an apparently good cream content and one apparently clean.

Sterilization is never advisable, and pasteurization is permissible only under unusual conditions. Some of the germs may be killed, but their poisons prevail. The sugar is changed to caramel, the taste of the milk is altered, there may be some coagulation of the proteids, the enzymes are destroyed or altered, and constipation usually results. Scurvy is caused no less by sterilized milk than by the proprietary foods. Fresh milk, ice, and cleanliness are points which need emphasis. Mechanical filtration through cotton is never a safe procedure. It is best, when possible, to arrange with some honest dealer for the milk of some certain cow or herd, and to insist on cleanliness, being specific as to directions and, if necessary, paying more for this milk; but even then certain analyses may be considered advisable.

Amount Used Daily.—The laboratory man must know the probable amount of milk necessary for the day's feeding, which is usually determined with ease. In case the babe has never taken artificial food, an estimate may be made and the formulas subsequently modified according as to whether the amount is too large or not sufficient. The amount will vary with the age of the child, the number of feedings daily, state of health, etc.

Duties of the Physician.—If the physician does not do the actual work, he will write the prescription to suit the case, and see that a sample of the milk reaches the laboratory.

Duties of the Analyst.—Each physician, as has been stated, may do his own work, or at least one physician in each community should be prepared to make the tests and estimations. The expense of equipment is small, and the technic as described in this work is exceedingly simple.

1. General appearance of the milk as to color, odor, amount of cream, etc.

2. Specific gravity.

3. Reaction.

4. Microscopy.

5. Tests for chemical purity. (When searching for germs, reference may be made to Searching for Germs, page 37.)

6. Quantitative estimation of fat content.

7. Modification, which consists of devising the amounts of cream, milk, water, and milk sugar to be used, bearing in mind the following factors:

- (a) Composition of the sample as determined by the tests.
- (b) The physician's prescription.
- (c) Amount to be made up daily.
- (d) Certain stock formulas which experience has shown will give mixtures that, when subjected to analysis, show the desired composition.

A study of the forms below, being similar to those used in hospitals, will give an idea concerning the meaning of the above arrangement. The first is a prescription written for a healthy baby which is denied the maternal milk, followed by the report of directions, etc.

R	Fats	2 percent.
	Carbohydrates	6 percent.
	Proteids	1 percent.
	Amount daily	20 fluidounces.

Report. Milk normal, the cream containing 16 percent fat.

Modification. As follows:

Cream	1 $\frac{2}{3}$ fluidounces.
Lactose	1 ounce.
Milk	3 $\frac{1}{3}$ fluidounces.
Water	15 fluidounces.

The above milk is mixed by the family. It is made up from cream, milk, and water to the 20 fluidounces and then the sugar is added. It will be remembered that when a solid dissolves in a liquid, the bulk is not perceptibly increased.

Analysis of Cow's Milk.—*Color.* This should vary from a white to a bluish tint, according to the amount of cream. Certain abnormal colors may be due to bacteria. The color of the collustrum is yellow, and needs no comment. The following arrangement will aid in the classification of such milks:

Color.	Etiological agent.
Blue	Bacillus cyanogenus.
Violet	Bacillus violaceus.
Pink	Bacillus prodigiosus.
Red	Bacillus lactis erythrogenes.
Yellow	Bacillus synxanthus.
Gelatinous color and consistency.....	Bacillus lactis viscosus.

Blood may color milk from a bright-red to a dark-brown.

Odor. Abnormal odors are rarely due to added preservatives, as these occur in very small amounts. The gelatinous milks may possess a very offensive odor, and, strange to say, the most disgusting of these cause no symptoms when ingested, whereas an apparently perfect milk may teem with death-dealing microorganisms or contain formaldehyd or borax in very small quantity. Certain vegetables, as turnips, cause a distinct and characteristic odor. A bitter milk may be explained by the presence of certain bacteria, purposely added poisons, etc. Milk absorbs odors, especially when stored in ice chests with melons, cucumbers, canned salmon, etc.

Amount of Cream. In a regular pint bottle which has stood over night the upper cream layer should by linear measure make up about one-third of the entire amount.

Specific Gravity. This may be taken with an ordinary urinometer and should average 1.030. In case it is much more, there is sufficient reason to suspect that cream has been removed. If it is considerably less, the milk has in all probability been watered.

Reaction. Milk is acid when tested with phenolphthalein solution. It is practically never alkaline unless putrefactive changes are present or chemicals have been added. Acidity is increased as the milk sours.

*Microscopy.*¹ If the cow is healthy, only a few leukocytes and epithelial cells may be present in her milk. Leukocytes in considerable number point to infection.

Detection of Formaldehyd. To 4 drams of milk in a test tube add 1 dram of commercial sulphuric acid. Do not mix, but permit the acid to form a layer below the milk. A violet ring at their junction is proof that formaldehyd is present. This test is exceedingly simple and delicate, and but two precautions are necessary:

¹ Experts are able to obtain merely from a microscopical examination considerable information as to the probable percentage of fat. A granular tendency of the droplets, or when these are much too large, points to a paucity of fat.

1. The reaction depends on the presence of iron contained in the commercial sulphuric acid; hence, if pure sulphuric acid is used, two drops of ferric chlorid should be added to it.

2. A pseudo-reaction may occur if acid is concentrated, or the charring of the milk may obscure the true reaction; hence an acid of about 1.700 specific gravity—i. e., slightly diluted—should be employed.

Detection of Borax and Boric Acid. Mix in an evaporating dish 1 dram of the milk with 1 dram of the fresh tincture of turmeric and heat, evaporating slowly to dryness. Add 2 or three drops of hydrochloric acid (dilute) to the residue and evaporate to dryness once more. Pink or red colorations are positive indications, and a drop of ammonia water should change either to a green. This test takes some time, and is rarely necessary as a routine procedure. A milk which shows no formaldehyd and which refuses to sour is likely to contain borax or boric acid. The chief source of error is too rapid evaporation by heat from the free flame. A good substitute for the water bath is to place the evaporating dish on the flat lid of a kettle of hot water.

Detection of Sodium Bicarbonate. Its probable presence may be determined by adding a drop of any inorganic acid—for example, nitric acid. In case soda is present, gas will be given off and the surface of the milk will froth. In case other alkalies are added, a drop of any chemical indicator will show at once the nature of the deception. The value of these tests would be limited in a decomposed milk.

While it is true that tests for chemical preservatives need not be made in all cases, yet positive reactions are often found where they might be least expected. Such poisons as have been mentioned are not usually added to milk during the colder months.

Quantitative Estimation of Fat. This is necessary with all creams, except perhaps those in which previous test has shown a



Fig. 32—Tube for fat estimations which may be used with an ordinary centrifuge.

definite percentage of fats. For a few cents a special tube (Fig. 32) may be purchased which may be used with the ordinary centrifuge. A mixture of 1 part of cream and 4 parts of water is added with a pipette to the 5 cc. mark. Add 1 drop of the Leffman-Beam solution, made up as follows:

R Hydrochloric acid	50 drops.
Methyl alcohol	13 drops.
Fusel oil	37 drops.

Mix by shaking, and then add concentrated C. P. sulphuric acid **drop by drop, shaking constantly or rotating tube until the zero mark is reached.** Centrifugalize five minutes and then read percentage of fats directly from scale. Multiply by five, which gives the percentage of fats in the sample of cream.

Formulas.—These are stock formulas, and their use has been previously considered (page 133). The following letter symbols and formulas are recommended for the home modification of milk:

Q—Quantity desired for twenty-four hours' feeding.

F—Desired fat percentage in modified milk.

S—Desired sugar percentage in modified milk.

P—Desired proteid percentage in modified milk.

FF—Percentage of fat found in the cream by analysis.

C—Amount of cream to be used in the prescription.

M—Amount of milk to be used in the prescription.

W—Amount of water to be used in the prescription.

SS—Amount of milk sugar to be used in the prescription.

Formulas for Milk Modifications.—

$$C = \frac{Q}{FF - 4} \times (F - P)$$

$$M = \frac{Q P}{4} - C$$

$$SS = \frac{(S - P) \times Q}{100}$$

$$W = Q - (M + C)$$

Example. Taking the above formula as a basis, substitute for it a 20-ounce mixture that is to contain 2 percent fats, 6 percent

carbohydrates, and 1 percent proteids, and where the cream has shown 16 percent fat.

$$C = \frac{20}{16-4} \times (2-1) = 1\frac{2}{3} \text{ fluidounces.}$$

$$M = \frac{20 \times 1}{4} - 1\frac{2}{3} = 3\frac{1}{3} \text{ fluidounces.}$$

$$SS = \frac{(6-1) \times 20}{100} = 1 \text{ ounce.}$$

$$W = 20 - (3\frac{1}{3} + 1\frac{2}{3}) = 15 \text{ fluidounces.}$$

It will be noted that "Q" is made up by the addition of the computed quantities of cream, milk, and water respectively. The milk sugar dissolves without increasing the bulk to any extent.

Less Frequently Applied Procedures.—These include mainly the bacteriological tests. Dr. Hutchinson's Manhattan in a spoon consists of as many nationalities as does the real Ghetto, from which it may be seen how impossible it would be for the general practitioner to isolate and study this population.

Analysis of Maternal Milk.—A search for a pathological leucocytosis or an estimation of fat may be seldom attempted by the practitioner, but, with these possible exceptions, little practical information is to be gained from a study of this secretion. It should be understood that some of the germs of an acute infection may pass into the milk, and that a laboratory examination will not be necessary to condemn it.

The questions of how to overcome the difficulties that may be met and the value and limitations of certain procedures have been previously considered in descriptions of the technic.

CHAPTER XII.

SOME SIMPLE WATER ANALYSES.

Apparatus.—This is listed preceding each test, and necessarily depends on the selection of the tests made by the analyst.

These tests, when compared with those procedures usually described, are simple. The recommendations made to the general practitioner may be criticised, but a careful study of the matter will show that the analysis by the chemist at a distance is not in every case perfectly satisfactory. In order to avoid any unfair interpretation of the value of these investigations, the authors ask that a careful study be made of this chapter. In all tests, only the water used for drinking purposes is considered.

Scientific Versus Practical Analyses.—A practical examination of drinking water is in all cases useful and in some instances indispensable. A full and scientific investigation will reveal certain facts that are as yet of little or no real value, and do not receive consideration in this book. It follows, therefore, that any criticism of the methods that are recommended can not properly be offered by individuals interested commercially in the "perfected water laboratory." It is not the intention to recommend, without limitation, every investigation mentioned in this book, for in this matter, as in other lines of work, a certain amount of practice is necessary. It may be advisable, in turning over the practical examination of drinking waters to the physician, to state two propositions and then to attempt their proof:

1. The physician may be able, after a little practice, to condemn a drinking water quite as quickly as an expert.

2. No chemist or bacteriologist can, from the examination of a water sample, recommend it for drinking purposes, and this applies to the expert as well as to the practitioner. That a water is probably safe can not be determined any quicker by the extended scientific examination than by carrying out the work as here indi-

References.—Jordon: General Bacteriology; Prescott and Wilson: Elements of Water Bacteriology; Harrington: Practical Hygiene. Very few books issued prior to the past two years bring this subject down to date.

cated. It should be remembered, however, that in this chapter is considered only a suspected water, and not the larger sanitary questions—not, for example, the typhoid epidemics, **where milk or flies may be the carriers**, and where expert assistance is always demanded.

The first proposition may be proven by the actual selection and description of the tests, their limitations, etc., together with a thorough study of these points by the physician and actual practice with samples.

The second proposition may seem more startling, and demands discussion at once. Unfortunately, for analytical purposes, the nature of the bacterial contamination of water is of much more importance than its actual amount. A water which yesterday contained the germs of typhoid may show none today, and these same germs may be found tomorrow or even immediately after the samples were taken. The water may be sparkling, clear, and without odor, and a careful chemical examination may reveal the minimum amounts of salts resulting from organic decomposition. A thorough bacteriological examination may neither show evidences of sewage contamination nor may it identify specific pathogenic microorganisms. The fact, therefore, remains that no laboratory analysis can at present recommend for drinking purposes any certain water.

Analysis of Commercial Waters.—Purgative waters—those recommended for “kidney disease,” “rheumatism of the young man,” “health waters,” etc.—are not considered in these tests. They contain large amounts of mineral water derived from deep-seated natural deposits, and in most of them the saline purgatives abound.

Detection of Poisonous Chemicals.—These are rarely found in drinking waters. Some western rivers have been said to contain in solution small amounts of arsenic and antimony (Vaughan). River water may be contaminated by certain discharges from factories—as, for example, a river near a chemical manufactory may be so poisonous that all fish in its water may die. Chemical poisons may be purposely thrown into a well or a stream, and in this manner families or even portions of armies have been affected. The method of detecting these poisons will be found in *Detection of the Common Poisons*, page 85.

Changing Water.—Various gastrointestinal disturbances may result from drinking a “new” water, caused, when that water is

pure, by mineral salts occurring in amount different from that to which the alimentary tract is accustomed.

Goiter Waters.—The authors have had opportunity to investigate so-called goiter waters in two different localities, and are convinced that the mineral theories are not well founded. One of these villages obtained its water almost directly from the Mississippi river. No specific amebæ were found in any of the many samples examined. The goiters occurred only in women who had spent most of their lives in the town, and in several of these cases were found typical symptoms of Basedow's disease. In no instance was the condition apparent before the age of 12 to 14 years, but this "goiter of puberty" usually remained throughout life. All women born in the village were not affected, but one who had come to the place when 45 years of age developed symptoms at 70.

Odor of Waters.—The once popular notion that the water with no odor was safe for drinking purposes has given place, seemingly, to the idea that a foul smell is necessary for a safe and "healthy" water. A bad odor may indicate sewage contamination or the presence of dead toads, rats, moles, etc., but beyond this has little to do with the purity of the water. Other causes of odors are:

1. Pig-pen and grassy odors are due usually to green algæ of shallow warm waters.

2. Geranium, oily, and fishy odors are due to certain other algæ.

3. Musty odors are observed sometimes in sewage contamination, but are due usually to certain molds.

4. A hydrogen sulphid odor is due usually to the action of certain bacteria on the sulphates.

5. Putrefactive odors are due not only to dead animals, but to decaying vegetable matter in stagnant pools or wells. (See page 141.)

Reaction of Water.—This is usually so variable as to be of little practical import.

Detection of Lead.—Symptoms of plumbism may direct suspicion to the water supply. If lead pipes are used and several people become ill, incrimination of the water is almost inevitable. To 8 ounces of the sample add $1\frac{1}{2}$ grains of potassium bichromate. A turbidity should result, which in twelve hours will show as a slight precipitate on the bottom of the glass vessel. It is best, when making this test, to set the vessel on an intensely black back-

ground and observe it at different angles. Control tests with distilled water and those containing traces of lead should be made.

Animal Parasites in Water.—Pinworms and roundworms are often distributed by water. The *bothriocephalus latus* is spread by the fish of the Baltic, and has been seen in several American clinics.¹ Other forms of tapeworm, trichina, and the several varieties of the vermes are not usually spread by drinking water, but many of the tropical worms are spread in this manner.

Dead Animals in Water.—The diagnosis of this condition is not usually difficult, and the physician is rarely consulted. A draining of the reservoir may, however, show no traces of dead animals, but the odor may nevertheless persist. Such a water supply need not for that reason be condemned, as putrefactive odors arise from the anaerobic decomposition of vegetable matter, which points at once to stagnation. Connecting the pump nearer the bottom of the well or filling up the stagnant space with sand will usually solve the problem. (Harrington.)

Algæ in Water.—Vegetable life under water performs the same functions as do the higher forms on land, working over animal excreta into assimilable matter. If possible, the various algæ should not be disturbed, as it is an established fact that they will destroy sewage bacteria. Some algæ give off obnoxious odors, and may cause various gastrointestinal symptoms. The cause is not usually difficult to find, and has been mentioned in the preceding paragraph. Such algæ should not be destroyed by copper solutions, as is usually recommended, but proper measures should be adopted to avoid stagnation. When these small water plants receive plenty of oxygen, their presence is more desirable than their absence.

Isolation of the Typhoid Bacillus.—From what has been said it seems unwise for the practitioner to attempt any procedure so difficult and so discouraging as the isolation of specific pathogenic bacteria. The typhoid bacillus has been identified in suspected drinking waters by Kübler, Neufeld, Fischer, Flatau, and others. In the light of our present knowledge an examination for its source is hardly justifiable, save by the expert as a part of a complete

¹Dr. A. S. Warthin has found the *bothriocephalus* meale in Lake Superior burbot. Indigenous cases of *bothriocephalus* infection in man have been observed in northern Michigan. See Michigan State Board of Health Report, 1912.

water analysis. Much work is to be done along this line, and a suspected water can be recommended for drinking purposes only when the analyst can say, "There are no typhoid germs in this water, and under present conditions none can find their way into it."¹ Such a recommendation, coming, however, from an assistant in some water laboratory many miles away and from the examination of a single sample, is nothing less than criminal.

Sewage Contamination.—On the other hand, a physician may condemn a water as quickly as an expert, provided he give the subject a little study and make analyses with sample waters, some of which are known to be pure and others to which he has added in small amounts sodium chlorid, feces, urine, old manure, etc.

Sewage contamination may be proven by certain inferences deduced from the sensible combination of two methods. Just which of these two has the greater value can not be proven by the counter arguments of either chemists or bacteriologists, but by proper conclusions from both. Neither is infallible, and either or both may, with certain restrictions, prove contamination by sewage, and the latter condition is sufficient to condemn any water. It seems advisable, in the first place, to eliminate certain examinations usually made in water laboratories:

1. *Odor.* May be noted, but it is well to remember limitations.
2. *Quantitative determination of certain chemical constituents resulting from animal refuse.* Where fecal contamination occurs, pollution by the urine must be coincident, and urea and sodium chlorid will be found. The decomposition of the former leads to the formation of various ammonia compounds, the amount of which will vary and of which quantitative estimations may be made with difficulty.² Their presence may be overlooked if the estimation of the extra chlorin may be easily made and if the value of this estimation be not excessively limited. (See page 143.)
3. *Certain other considerations of scientific interest, but of no practical importance.*
4. *Counting bacteria present.* This may have some significance when the presence of unusually large numbers of microorganisms

¹ The attention of any one interested in isolating the typhoid bacillus from drinking water is called to the process devised by Jackson and Melia, where a special agar-agar is used in its cultivation. An account of this method was presented to the American Public Health Association at Winnipeg in 1908. The process is fully described in Leffmann's "Examination of Water," January, 1909.

² To quote directly from Dr. Leffmann's preface to his recent (sixth) edition of his book on water analysis, we note an important conclusion, "The figures for nitrogen or ammonia are of much less value than is generally supposed."

is noted, but even this does not attribute any specific pathogenesis to any variety present.

5. *Determination of pathogenic species by animal inoculation.* This method of bacteriological water analysis, devised by Vaughan, is of the highest value when searching for the typhoid and colon germs, but can not be considered here.

Comparative Chlorin—Its Definition.—It is not sufficient to draw conclusions from the amount of chlorin in a given water, and this is one of the instances where a chemical laboratory at a distance fails to give valuable evidence. It is not enough to make a quantitative estimation of a certain water, but calculations should be made from other carefully selected waters, at least five in number, representing all possible sources within a radius of ten miles. A careful study of the question will show the necessity for such a precaution. It would be manifestly unfair to condemn a water in certain localities containing much chlorin. The ocean may not be far distant, and, moreover, such condition might be explained by certain geological formations. An analysis of a water one mile distant might give similar results, and it is, therefore, not the actual chlorin increase, but the comparative chlorin content—as compared with other waters in that section—of the sample which determines its safety. Two wells within a few rods of each other may each show large amounts of chlorin, and the question will be whether both are contaminated by sewage. Another well a quarter of a mile away may show considerable less salt, as geological formation is not so sharply defined. Samples should, however, be taken in other directions before conclusions are attempted, and, if the chlorin content of these falls short of those noted in the first two wells, it is quite probable that the latter are contaminated; if so, compare with results of bacteriological investigations on page 146.

Comparative Chlorin—Its Estimation.—At least four waters besides that suspected should be examined before an opinion is given. As the physician may more easily obtain such samples than the laboratory at a distance, his opinion will be of greater value. A careful selection of these samples may be difficult, but none the less imperative. “Level” comparisons as well as “distance” comparisons are of value, the method of choosing depending on the principles outlined above. A study of the geological survey of the region in question may be advisable. The results obtained from

an investigation of the first set of samples may call for an examination of other waters before a final conclusion can be reached.¹ The following apparatus and reagents are necessary:

- | | |
|--|---------------------------------------|
| 1. Silver nitrate solution. ² | 4. Piece of white paper. |
| 2. Indicator. ³ | 5. Glass stirring rods. |
| 3. Two large tumblers or beakers. | 6. Buret as used in gastric analysis. |

Place the two beakers side by side on a piece of white paper. Add to each exactly 100 cc. of the water and 10 drops of the indicator. The silver nitrate solution is added drop by drop from the buret into the beaker until a slight red tint is noted in the water, when it should be compared with the control. This titration is conducted exactly as in the gastric analysis, each drop being stirred into the water and the reaction given time to take place. After a little practice, less than five minutes will answer for the titration of each sample. Readings are taken on the buret immediately preceding and following the titration. The amount in cubic centimeters of the silver nitrate indicates the number of milligrams of chlorin in the water.

Example.—If 2.5 cc. of silver nitrate are used for the 100 cc. of water, this sample contained 2.5 milligrams chlorin (which is a very good average in some regions).

Now titrate in the same manner the other samples, and compare results as advised above. Does the sample contain too much chlorin—**too much chlorin as compared with other waters of that region?**

Comparative Chlorin Estimation—Principles Involved.—The final red color is caused by the chlorides using up the silver solution as long as they are present. A somewhat less affinity is shown by the indicator, so that an excess of the reagent causes a formation of red silver chromate and proves that the chlorin has been consumed.

Sources of Error.—The “end reaction,” or the first red tint, may be difficult for the beginner to recognize. In case there is a question, take the reading and then add 1 or 2 drops of the silver

¹ When the worker becomes sufficiently acquainted with the characteristics of the drinking waters in his vicinity, and has determined an average chlorin or a standard, it will not be necessary in every instance to run control tests.

² One liter of distilled water contains 4.797 grams of chemically pure silver nitrate, and 1 cc. of this solution is equivalent to 1 milligram of chlorin.

³ One hundred cc. of distilled water contain 5 grams of pure potassium chromate. Add some of the silver nitrate solution until a red precipitate forms, and filter. This solution serves as indicator. Both solutions must be made up by some reliable firm or pharmacist.

solution. In case the end reaction is present, the mixture will become very red, and it is a good plan to try several "knowns." For example, if 2 milligrams of sodium chlorid are added to distilled (chlorin free) water, exactly 2 cc. of the silver solution should cause the red tint, etc.

Value of This Estimation.—From a practical standpoint, this computation is of great value. Although a scientific analysis would consider ammonia, nitrites, and several other estimations, it rarely takes into account the comparison of the different drinking water sources. As contrasted with the tests to follow (page 146), chemists and bacteriologists disagree. Neither is infallible, and each helps the other. It is a question of personal application, and the man who makes both, and then **skillfully** interprets results, gains much more than he who spends hours in the determination of hardness, residue, and total number of bacteria, and who in the end can not conscientiously attempt a conclusion.

Limitations of This Estimation.—The estimation of chlorin forms a routine procedure in most water laboratories, and the comparative method as described above is not **always** used. The authors have used it successfully in several problems where bacteriological examinations appeared to be of secondary consideration. So far as the simple estimation of chlorin is concerned, and without reference to other waters of the region in question, the limitations are so many as to render the value and rewards of such labors practically nil.

Bowel Bacilli—Definition.—Some bacteriological method should be used in connection with the chemical procedures. Of these there are several which, with certain limitations, may be employed advantageously by the practitioner. A single principle is involved in their application. Where fecal contamination is present, we should, without much difficulty, identify certain microorganisms whose normal habitat is the bowel. The possibility that these come from the alimentary tract of other animals has no bearing on the question—a solution of manure was never intended for drinking purposes. The colon bacillus, at least, is not a normal inhabitant of the gut of a fish (Amyot), and it is the identification of this microorganism that indicates contamination by sewage.

Identification of Colon Bacilli.—Many methods have been devised, and all have limitations which render them of less value than may be desired, yet, when skillfully interpreted, form a valuable

adjunct to the comparative chlorin estimation. Two of the many methods are presented, with respective cautions concerning interpretation of results, and either or both may be used:

Litmus and Gas Method—Apparatus.—

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|--|--|
| 1. Sterile glucose agar-agar in tube.
(See Searching for Germs, page 38.) | 2. Azolitmin, sterile 1-percent aqueous solution. ¹ |
| | 3. Platinum loop. |

METHOD. The tube is inoculated with about 2 cc. of the sample water according to the methods described in Searching for Germs, page 38. The agar is liquefied and the sterile azolitmin added and stirred in, about 5 drops of the latter being usually sufficient. When the temperature of the water bath sinks to 120° F., the inoculation must be quickly made. Care should be taken to avoid contamination by following the precautions given on page 39. With a sterile platinum loop mix well the sample with the rapidly solidifying media and keep at body temperature for twenty-four hours. Reddish colorations or evidences of gas formation, usually both, may be caused by the colon bacillus.

This test is not absolutely conclusive as to the presence of the colon bacillus, but is valuable when used in connection with the comparative chlorin estimation. The tetanus bacillus may cause gas formation, though this hardly occurs within the twenty-four hours. The typhoid bacillus may cause the red coloration, but this would not argue in favor of the water.

Fluorescence and Gas Method—Apparatus.—

- | | |
|---------------------------------------|--|
| 1. Sterile glucose agar-agar in tube. | 2. Sterile 1-percent solution of neutral red. (Grübler.) |
|---------------------------------------|--|

METHOD. This is a very valuable method, and in a large majority of cases, when positive, points conclusively to contamination by the colon bacillus. Several anaerobes give the reaction, but the typhoid bacillus does not. The technic is identical with that used in the litmus test, except that 2 drops of the neutral red solution are used instead. The colon bacillus produces in this media not

¹ Certain precautions are necessary when attempting the sterilization of azolitmin and other delicate chemicals, as continued boiling results in their partial disintegration, and azolitmin may be decolorized, although some of the original color may be regained. The colon bacillus should not be present if pure reagents and sterile water are used, and neither of these should be contaminated by handling, but a little heating is the safest method. It forms no spores, and is usually killed below 170° F.—i. e., a temperature considerably short of the boiling point.

only gas, but a beautiful lemon-yellow color, with a green fluorescence. The fact that the bacillus of hog cholera might respond to this test does not indicate that such a water should not be condemned.

Conclusions from a Bacteriological Examination.—Many other methods have been proposed both for the identification of the colon bacillus and for other microörganisms characteristic of sewage, but, used alone, none of them are of much value. When, however, the comparative chlorin is high, and when either or both of these bacteriological tests are positive, the physician may safely condemn the water, and err less frequently than on many other diagnostic questions. When the chlorin corresponds with that in other wells of that locality (see Limitations, page 145), and bacteriological tests are negative, then he may say, "This water is **most probably** safe for drinking purposes."

Conclusion.—The authors believe that they have proven their two propositions, and recommend these examinations to the physician. In case assistance is necessary, the expert should not be sent a sample of the water, but should be called to the "seat of action," and he should also be called at once in regard to the more important sanitary questions previously mentioned (page 139).

A full and scientific examination of a water usually confuses. A sample for a practical analysis should not be sent to a laboratory over a hundred miles away for the following reasons:

1. Certain bacterial changes occur during transportation, even where ice is used. (Jordon and Irons.)

2. An investigation of a single sample by a distant laboratory is subject to so many limitations that it is of but little or no practical value.

CHAPTER XIII.

EVERY-DAY STOOL TESTS.

Apparatus.—

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|--------------------------|--------------------------------|
| 1. Acetic acid, glacial. | 5. Glass stirring rod. |
| 2. Acetic acid, dilute. | 6. Hydrogen dioxid. |
| 3. Benzidin. | 7. Microscope and accessories. |
| 4. Evaporating dish. | |

Stools are generally so offensive that their examination in private practice is often discouraging, and is never necessary as a routine procedure. Certain symptoms may, however, direct attention to the matter, and it is then that an inspection or a chemical test may mean much in a diagnosis. A full and scientific fecal analysis is rarely of use outside of the hospital. The physician dreads, and with sufficient reason, a necessary test, but an elevated nose never diluted any gas. The pathological stool is not usually vile smelling, while that of the person without ills is almost unbearable. Many interesting examinations have been omitted simply because they are not, so far as the practitioner is concerned, every-day tests.

Obtaining the Specimen.—A mere inspection of the entire stool is sometimes sufficient—as when looking for large worms, examination of infants' stools, etc.—but for chemical and microscopical tests small samples containing mucus or bits of blood are selected, and these may be carried to the office in a tightly corked bottle, the mouth of which is sufficiently wide to permit the entrance of instruments. If a search is to be made for the ameba coli, the specimen must be kept warm until examination of the living animal is completed. Bits of material may be transferred to the test tube or slide with a pipette or platinum loop. Such examination may be made after office hours, and suitable methods of deodorizing employed. (See page 194.) Smears, after fixation, give off no odor,

References.—Sahli, Wood, Boston, Webster, Simon, and other works on clinical diagnosis.

and may be examined the following day in case good artificial light is not available at the time.

Odor.—This is of little diagnostic import. There may be little or no odor in starvation and certain chronic diseases, but the odor of a meat diet is most marked.

Color.—The following table gives the colors most likely to be met in the stool of the adult:

Color.	Etiology.
Brown, various shades.....	Normal.
Dark-brown	Meat diet.
Gray or clay-colored.....	Obstructive jaundice, fatty diarrhea, etc.
Light-yellow	Exclusive milk diet, large amounts of starches, santonin, rhubarb, senna.
Red	Fresh blood, hematoxylin, in frauds.
Tarry-black	Blood.
Granular-black	Bismuth, iron, and manganese.
Green	Calomel, spinach, lettuce, parsley, infections with bacillus pyocyaneus, etc.
Blue or green on standing.....	Methylene blue.
Violet	Santal oil.

Consistency.—The consistency of an adult's stool is so variable as to be of little value in diagnostic procedures, and depends mainly on the amount of water present. For the differentiation between pus and mucus see *The Sputum*, page 27.

Test for Occult Blood.—Dissolve about 1 grain of benzidin in about $\frac{1}{2}$ dram of glacial acetic acid. Stir into $\frac{1}{2}$ dram of dilute acetic acid about 2 grains of the feces, and to 3 drops of this resulting liquid add 20 drops of 3-percent hydrogen dioxid and 20 drops of the benzidin solution. Stir the mixture, and, if blood is present, a green or blue coloration soon results. This will identify very small amounts of blood. Bleeding adenoids must be ruled out, and foods containing meat should not be allowed for several days preceding the test. It is usually a waste of time to attempt the identification of fecal blood by the presence of red corpuscles unless they occur in very large amounts.

Searching for Bacteria.—As a routine procedure, this is of little practical value because of the enormous numbers and varieties of germs normally present.¹

¹ Of these enormous numbers it seems safe to say that over 75 percent are dead and 20 percent are attenuated or incapable of growing rapidly in the feces. A few, however, notably the colon bacillus, may cause widespread mischief when given a chance.

Searching for Ameba Coli.—The sample must be kept warm, as it is often difficult to identify the amebæ, except by the motions of their pseudopods, and this activity ceases below 75°–80° F. The protrusion of a pseudopod is not a rapid process, and it is not usually observed with the eye, but with the aid of a series of rough drawings, one of which is taken every thirty seconds. A comparison of these may show that a change in shape has actually occurred. The amebæ may often be differentiated from epithelial cells on account of their light-green tint and because they are likely to contain leukocytes, bacteria, particles of food, etc., in the cytoplasm.

Detection of Koch's Bacillus.—The diagnosis of intestinal tuberculosis by the identification of the specific bacillus is subject to various limitations, as tuberculous sputum may be swallowed, and, unless clinical symptoms are sufficient to justify such an examination, conclusions may be very misleading. As in the pulmonary form, there is a presloughing stage, when few or no bacilli are loosened into the lumen of the intestine. In acute miliary forms death may occur before any ulcers have formed. A positive test for occult blood may be the first sign that the mucosa has become necrotic, although bacilli usually begin to appear in the feces at this time. There are also normally many acid fast bacilli in the stools of an adult, notably the timothy bacillus, and these may be differentiated from Koch's bacillus by the use of the hydrochloric alcohol solution. (See The Urine in Disease, page 123.) The tubercle bacillus is most likely to be found in the mucous, purulent, or blood-stained particles. For its identification see The Sputum, page 32.

Searching for Pinworms.—These may be visible at the anus, clinging to the lowest mucosa or hairs, and have the appearance of long white crystals of hoarfrost. The first bowel movements are most likely to contain them, but never contain their ova. They give rise to local disturbances, and may usually be identified without the use of enemata.

Searching for Ascaris Lumbricoides.—This occurs in the small intestine, but is rarely passed, as it holds fast to the intestinal wall by means of spiral turns. When found, it may be identified by its smooth appearance and its light-brown or flesh color. It is usually the length of an ordinary lead pencil, and tapers to points at both ends. Its ova are often found in the feces, and are elliptical in

form, with a hard shell, which is surrounded by an albuminous covering.

Many physicians desire to save specimens of these worms, and the following technic will give the best results: Kill in 70-percent alcohol heated to about 175° F., permit to cool, and transfer to preserving fluid made up of 70 parts absolute alcohol, 5 parts glycerin, and 25 parts distilled water.

Searching for Tapeworms.—Segments frequently occur in the feces. The ova are globular and are covered with thick capsules. It would seem that the morphology is so characteristic that the

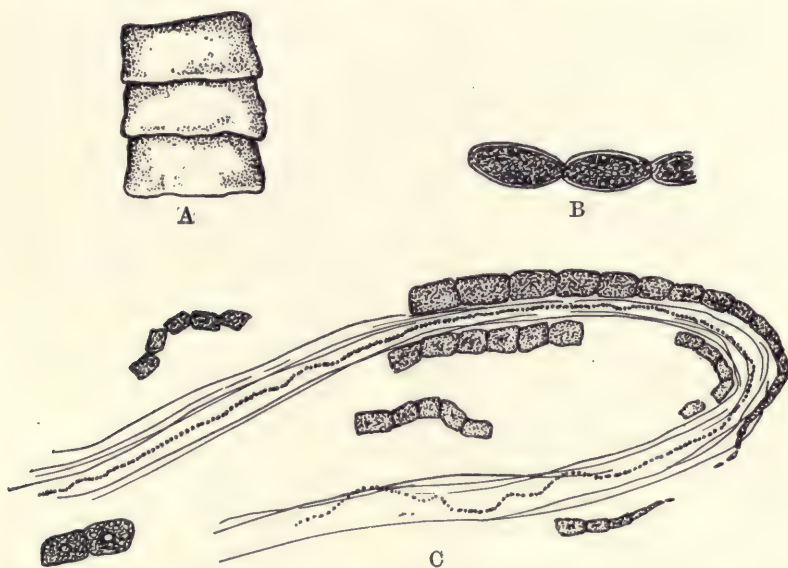


Fig. 33.—Tapeworms compared with vegetable fibers. A, B, magnified segments of small tapeworms; C, magnified banana fibers, recovered from stools of an infant.

confusion of tapeworm segments with other elements would be impossible, and it is safe to say that a tapeworm is rarely mistaken for anything else. It is, however, often surprising to find how closely certain vegetable fibers resemble the segments of small teniæ, of which the banana furnishes an example.

Tapeworms may be killed and preserved as follows: Wash in water, being careful not to break the worm. Kill in equal parts of a saturated aqueous solution of mercuric chlorid and 70-percent alcohol, to which has been added 1 percent (of the entire volume)

glacial acetic acid. Heat to 160° F. and cool. Wash gently in running water, and remove excess of mercury with iodine alcohol. Rubber gloves should be worn to prevent cysticercus infection. Specimens may be preserved as described for *ascaris lumbricoides*, page 151.

Tapeworms may be stained and mounted on slides by the following method: After killing, flatten gently between two slides held together with a rubber band, and stain in carmine about six hours. Remove from between slides and decolorize in alcohol, occasionally examining the specimen under low-power objective until a suitable picture is obtained. Transfer to 95-percent alcohol and then to

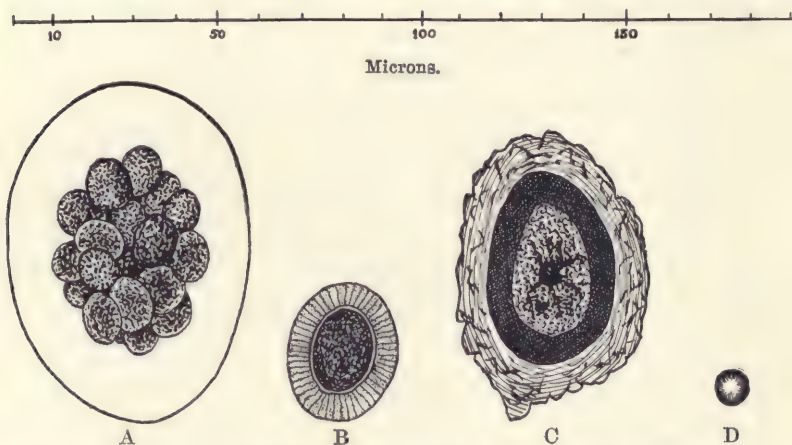


Fig. 34.—Ova of the most common intestinal worms compared with a red blood cell. A, ovum of hookworm, thin shell containing segmenting or segmented material; B, ovum of tapeworm, thick horny shell; C, ovum of round worm, thick shell surrounded by albuminous capsule; D, erythrocyte.

absolute alcohol in order to remove water. Clear in carbol-xylol and mount in balsam. If aqueous carmine rather than the alcoholic solution is used, it will not be necessary to compress specimen until after decolorization.

Searching for the Hookworm.—The adult parasites are sometimes found in the stools, but generally a search must be made for the ova, which are usually discharged as segmented masses, resembling a small bunch of large grapes inclosed within a thin shell. In cases of "southern anemia," where neither hookworms nor their ova are found, a test should be made for occult blood according to previous directions (page 149). This condition is caused

by the hemorrhage of the mucosa continuing after the hookworm has departed to some other portion of the gut.

Comparison of the Common Ova.—There is hardly any need for contrasts when the symptomatology, geographical distribution, and other factors are considered. Ova of worms are transparent or of a brown color when within the body of the female parasite, but when passed by the bowel are yellow or brown because of the urobilin present. The smallest of these has a diameter three times that of the red blood cell, while many are even larger than epithelial cells. They should not be confused with the latter because of certain characteristics that are absent in all epithelial cells, but some of which may be seen in any of the ova. These characteristics are their elliptical form of almost geometric regularity, presence of a capsule which may in some of the eggs become quite thick, and tendencies to segmentation within this shell.

Fecal Findings in Several Conditions.—

1. *Starvation.* Little or no odor; formed stools.
2. *Vegetable diet.* Light-brown color, or even green when fresh vegetables are used.
3. *Milk diet.* Light-yellow color, with but little odor.
4. *Meat diet.* Dark color and very marked odor.
5. *Constipation.* The typical stool is very hard, dry, and often void of odor; shows a tendency to crumble. Curiously enough, watery stools are sometimes observed in costiveness.
6. *Mucous colitis.* Intestinal mucus occurs normally in small amounts, but is increased in most pathological conditions. As it is easily digested, it comes from the upper bowel only in cases of marked diarrhea. Mucus is usually jelly-like in character, but may appear as leathery masses, or even as casts of the gut. The particles may resemble pieces of asparagus or intestinal worms. The mucus of an inflammation is easily differentiated from that of a truly nervous condition, as the former contains inclusions of cells or food, while the latter does not.
7. *Internal injuries.* Surgeons watch with interest the first urine and feces of emergency cases for signs of blood.
8. *Typhoid.* "Pea soup" feces of an offensive odor, and containing in small amount pus, necrotic tissue, and sometimes blood.
9. *Tuberculosis, carcinoma, etc.* Blood, pus, etc.
10. *Dysentery.* "Watery" feces, containing necrotic tissue, amebæ, and blood.

11. *Cholera*. "Rice water" stools, which late in the disease may be practically a suspension of vibrios in blood serum.
12. *Lienteric diarrhea*. Much undigested food and little mucus.
13. *Colonic diarrhea*. Little undigested food and much mucus.
14. *Drug administration*. (See color of feces, page 149.)
15. *Obstructive jaundice*. The clay-colored stools are not due so much to the absence of bile pigments as to the great increase of undigested fats and soaps.

Stools of an Infant.—Within the first twenty-four hours the dark-brown or black meconium should be passed. The stool of a milk diet is unformed, of a butter-like consistency, and of a light-yellow color. There are many variations from this type, **none of which can be considered pathological unless symptoms of disease or disorder are present**, a point that is often overlooked. Any variations, however, from the typical milk stool **occurring in connection with illness** on the part of the infant become at once of the greatest diagnostic and prognostic importance. For example, a cheesy stool in a healthy infant means nothing, showing only an excess of undigested casein, and does not indicate drugs or diet changes, but, should a severe diarrhea accompany or immediately follow the appearance of these white, lumpy stools, the indication is then clear—barley water or a reduction of proteids. Keeping in mind this limitation, the following table is given:

Appearance of infant's stool.	Significance (morbid only when certain symptoms justify).
Pink streak	Uric acid infarct (contamination by urine).
White and cheesy.....	Undigested casein.
Gray	Obstructive jaundice, excess of fat.
Green	Changed bile pigments.
Curds, colic, with constipation or diarrhea	Proteids at fault.
Green or greenish-yellow, sour, with loose bowels	Fats or sugars, usually the former at fault.
Mucus	Malnutrition, or accompanies severe intestinal inflammation.
Red	Blood from lower alimentary tract.
Brown	Blood from upper alimentary tract.

An inspection of the napkin is usually sufficient, and an occult blood test may be occasionally indicated. Fatty acids may appear as lump-like masses, and are sometimes mistaken for undigested

casein. The former are, however, quickly dissolved by ether, which may be strained away from the stool proper, to reappear when the ether evaporates.

Tampering with the Stool.—The “worm faker,” in times past, reaped a rich harvest from his victims. When teniæ were not obtainable, earthworms, grubs, and centipedes answered equally well. A western “expert” used, with astounding success, a pickled spinal cord with its branches, and hundreds of grateful persons passed this single specimen. Eventually, however, skeptics began to find fault with the darkened bed-chamber, persons became enlightened, and vegetable fibers or other bits of extraneous matter were picked out and shown under a microscope. The claim has been made that certain drugs are capable of dissolving many of these worms within the bowel, but often the object is to deceive.

The pseudo-gallstone seems of late to have become prominent. Olive oil or similar fatty bodies are sent to the victim, who is urged to take the prescribed substance in large doses, and advised to make frequent examinations of the bowel discharges. He soon begins to find large soft and greasy lumps, which may be white, but are usually green if the bile ducts are not occluded, and consist of fats, fatty acids, and certain soaps. The dupe rushes wild-eyed to the local physician, who too often agrees that biliary calculi have been passed. A second thought, or indeed a brief study of the appearance of gallstones, would at once expose the imposition. Hematoxylin may color the feces a bright-red, and should cause no more concern than a methylene-blue-laden urine.

Less Frequently Applied Tests.—Certain dietetic tests and difficult bacteriological searches, as well as certain scientific examinations, are omitted, as a study of the various crystals and cells in feces may prove a waste of time to the practitioner.

Value and Limitations of Stool Analysis.—Many of these limitations have been pointed out, and in selected cases the value of certain searches is so well understood that emphasis is unnecessary. A routine stool examination in every case of sickness is worse than a waste of time to the physician. If, however, certain symptoms lead him to suspect the presence of blood or parasites, or the infant seems to be slowly starving to death, and he fails to make the few necessary investigations into the character of the stools, he ceases to deserve not only the name “diagnostician,” but also “therapist.”

CHAPTER XIV.

TECHNIC OF THE PRIVATE POST-MORTEM.

Equipment.—A complement of autopsy tools may be selected from the surgeon's hand-bag and a carpenter's kit, consisting of a cartilage knife or heavy scalpel, amputating knife, large shears, probe point shears, surgical saw, bone forceps, chisel, hammer, bone drill for wiring skull cap into place, and wire, sutures, needles, probes, tissue forceps, etc. A pair of scales and a foot-rule should be included if possible. The high-priced post-mortem set offers no advantages over this selection so far as the private examination is concerned.

Private Post-Mortem.—The complete autopsy is rarely conducted in private practice, and, so far as the various lines of research are concerned, the clinic will continue to offer greater advantages than may be obtained by the general practitioner, but this does not indicate that the private post-mortem, incomplete though it may be, is wholly without a science. An indiscriminate slashing may demonstrate but little, while a few well-selected, though crude, dissections may reveal all that is desired to be learned. No pathology can be included in this book, except so far as elucidation of details in technic is necessary. The description given is essentially that offered by Warthin and other experts in this branch of medicine, modified only where it is necessary to meet the needs of the average practitioner.

Precautions.—

1. Even though the relatives request the autopsy, a written permit is always necessary, and such permit may be given by the following persons in the order named: (1) husband or wife, (2) children, (3) mother, (4) father, (5) brothers, sisters, and (6) other relatives.

2. In case of uncertainty as to the cause of death, and if an autopsy is forbidden by relatives, the coroner is notified. In case

of absence of the latter, the physician, some courts have decided, may stop funeral preparations and proceed on his own authority to ascertain the cause of death, even though he must resort to an autopsy.

3. No unnecessary mutilation of the cadaver is allowable. If necessary, small bits of tissue may be reserved for histological examination.

4. The presence of unprofessional persons is debarred by law, but medical students may attend.

Such, briefly, are the usual legal precautions. In private autopsies, however, certain considerations should be shown the relatives and friends, who may be waiting for the funeral services to proceed, and these considerations may be briefly outlined as follows:

1. Provide for the disposal of blood and other waste without being seen by the mourners. Suspicious material should be thoroughly mixed with disinfecting solutions and buried.

2. Perform the work quietly.

3. Avoid odors by burning coffee, sugar, rags, or tobacco.

Preparations.—Gowns and rubber gloves should be worn if possible, and strict antiseptic methods followed. It is not advisable for obvious reasons to use the dining-room table. A work-bench, or some rough boards supported by chairs or trestles, answer very well. A canvas cot covered with oilcloth has been used, and blocks or bricks placed under the legs, but this support is not satisfactory when sawing the skull cap. The floor should be covered with several thicknesses of paper to avoid soiling. Plenty of cold, clean water should be at hand, and warm water is preferable when washing up after the autopsy. The cadaver should be entirely stripped if possible. For the treatment of wounds obtained during autopsies, see *Laboratory Prophylaxis*, page 179.

Bacteriological Evidences of Disease.—So much information may be obtained by certain examinations during life, that it is advisable that these be not deferred until too late to use the information in therapeutic procedures, as often nonpathogenic germs are already emigrating from the intestines into the tissues before the final breath of life leaves the body.

Microscopic Morbid Anatomy.—Minute inspections are often necessary to clear questionable points, and the microscope is then called into requisition. Small bits of organs may be dropped into

formalin or alcohol, and examined at leisure according to directions in *Essence of Tissue Diagnosis*, page 78.

Weights and Measures.—Increase of weight accompanies the congestion of various organs. Certain atrophies and hypertrophies may cause alterations, often strikingly apparent, but which may be evident to the nonexpert only by the proper use of scales and foot-rule. To obtain a dimension, thrust a hat pin through the desired portion of the organ, and read the result by applying the foot-rule. If desirable, measurements may be made with a pelvimeter. The following table, taken mainly from "Warthin's Protocol," gives average normal weights and dimensions, but the English system is used where possible:

Length of male cadaver	70	inches.
Length of female cadaver	61	inches.
Weight of male cadaver	2,344	ounces.
Weight of female cadaver	1,875	ounces.
Circumference of skull cap.....	20 to 30	inches.
Longitudinal diameter of skull cap.....	7.5	inches.
Transverse diameter of skull cap.....	6	inches.
Weight of male brain	42.5	ounces.
Weight of female brain	39	ounces.
Weight of male cerebrum	36	ounces.
Weight of female cerebrum	32.5	ounces.
Weight of male cerebellum	4.5	ounces.
Weight of female cerebellum	4	ounces.
Weight of spinal cord	About 1	ounce.
Length of spinal cord	About 18	inches.
Weight of male heart	9.5	ounces.
Weight of female heart	8	ounces.
Size of heart...Usually that of right clenched fist of individual.		
Size of mitral valve	Admits 2	fingers.
Size of tricuspid valve	Admits 3	fingers.
Size of pulmonary valve	Admits 1.5	fingers.
Size of aorta	Admits thumb.	
Thickness of wall of right ventricle.....	2 to 3	millimeters.
Thickness of wall of left ventricle.....	7 to 10	millimeters.
Weight of left lung	10 to 15	ounces.
Weight of right lung	11 to 16	ounces.
Weight of thyroid	1 to 2	ounces.
Weight of thymus at birth.....	$\frac{1}{3}$	ounce.
Weight of thymus at puberty (disappears after age 25) ..	$\frac{2}{3}$	ounce.
Weight of spleen.....	5 to 8	ounces.
Size of spleen	.5x2x1	inches.
Weight of liver.....	50 to 63	ounces.

Size of liver.....	10x12x4	inches.
Weight of pancreas.....	3 to 4	ounces.
Size of pancreas.....	9x2x2	inches.
Weight of adrenals	1 to 2	drams.
Weight of right kidney	4.5	ounces.
Weight of left kidney.....	Little over 5	ounces.
Weight of prostate	About 5	drams.
Weight of resting uterus.....	1 to 1.5	ounces.
Weight of gravid uterus	Very variable.	
Weight of virgin ovaries.....	3	drams.
Weight of adult ovaries	2	drams.
Cortex of kidney is about $\frac{1}{2}$ inch in thickness.		
Kidney glomeruli are red and about the size of pin-points, being slightly elevated above the surface.		
Spleenic follicles are about the size of a medium brass pin-head, gray, and not elevated.		
Uterus wall is about $\frac{1}{2}$ to 1 inch in thickness.		
Gall bladder wall is about 1 to 2 millimeters in thickness.		

Order of Procedure.—A systematic technic is desirable, and, in case only portions of the body are to be examined, the following order may be accordingly modified:

1. Inspection, palpation, and percussion.
2. Head.
3. Opening of trunk.
4. Heart is examined before the lungs in order to preserve the relation and position of soft clots, etc., which might otherwise be drained away.
5. Lungs.
6. Neck and thoracic vessels.
7. Spleen.
8. Kidneys and adrenals.
9. Stomach and intestines.
10. Liver.
11. Pancreas.
12. Great vessels.
13. Pelvic viscera.
14. Spinal cord. If only the cord is to be examined, it may be removed from the back (page 168).
15. Joints, lymph glands, etc.

Clinical opinions are of little worth so far as an actual gain in medical knowledge is concerned, and the autopsy should be approached with the expectation of finding every pathological possi-

bility. The circumstances of the case may not, however, permit such procedure, as the relatives may consent to an examination of only the kidneys for evidences of Bright's disease, etc. In suspected Addison's disease or gastric conditions the adrenals and stomach should be examined as early as possible, as autolysis often rapidly proceeds in these organs after death.

Inspection, Palpation, and Percussion.—Inspection serves to reveal not only the changes due to the cessation of the respiration and circulation, but may often show the cause and circumstances of death, or give other evidence in regard to the nature of the fatal disease. It may be too late for a complete physical examination, but a consulting physician may nevertheless be justified in hastily palpating and percussing the cadaver.

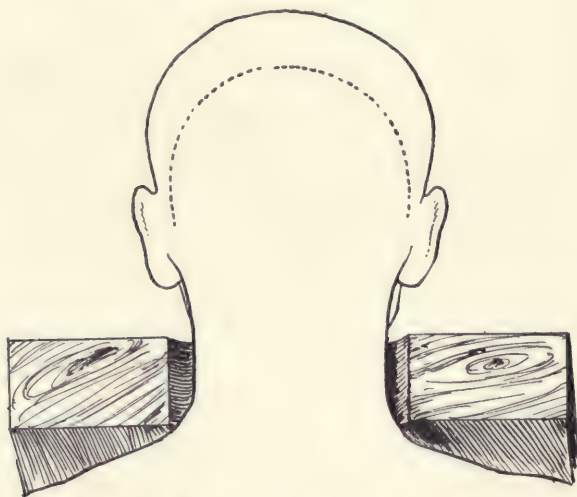


Fig. 35.—Scalp incision. The scalp incision must be made in the dorsum in order that it may not be visible at the funeral. It extends from ear to ear and completes the largest occipital circumference.

Uncovering the Brain.—The cadaver is laid on its back, and its hands placed under the buttocks in order to prevent their dangling over the edges of the table. A block should be placed under the neck or head in such a manner that the head is raised high from the table, the vertex pointing upward. The hair is wetted and parted in such a manner as to expose the line of the initial incision. The dorsum is selected for the incision in order that no marks can be observed at the funeral, extending from ear to ear and com-

pleting the greatest occipital circumference. If desired, the cadaver may be rolled on its belly while the incision is being made (Fig. 35), or, if left supine, the head should be raised as directed above.

The cut should extend to the periosteum, and may be made with scalpel or cartilage knife. The anterior flap is pushed forward and hooked under the chin. The origin of each temporal muscle is removed by a semicircular incision and peeled downward. The periosteum may be removed prior to opening the skull cap if so desired. During these operations and others which may follow the prosector should be on the lookout for evidences of disease, and hair, scalp, muscles, and periosteum should be subjected to a rigid inspection.

Some operators prefer to remove the entire skull cap by a circular cut. The angular cut, however, offers many advantages, and is recommended by competent authorities. The angular cap is more easily replaced in its natural position, and is less likely to present at the funeral a frontal line or joint beneath the skin. The angular cut is not nearly so difficult, and is much more cleanly because of the absence of hypostatic blood. It may begin at the forehead as a circular incision, but is discontinued at the aural lines, and from these points two posterior oblique cuts extend upward and cross just dorsal to the posterior fontanel, forming an acute angle (Fig. 36).

The left hand may anchor the head of the cadaver with the anterior flap, while the right hand uses the saw. A long stroke quickly minces the cerebral surface, affording but a short slit into the cranial vault, and it is therefore advisable to saw on the most convex surface of the bone. A very short stroke is imperative.

Unless the dura is greatly distended by fluid, or is adherent as in babes and the aged, there is no excuse for cutting it. A slight loss of resistance indicates the necessity of an exploratory probe. The sawdust is white, then red, and again white, as the tables are respectively reached, and the dura, when touched, may give forth a sound not unlike that of a dry, rustling leaf. When the probe indicates that the sawing has been finished, insert a chisel in the right temporal region, making a quick turn. The fingers are inserted and the piece of bone pulled back, the dura stripping slowly and always adhering at the longitudinal sinus.

Examination of Meninges.—To measure the intradural tension,

attempt to pick up one fold over the frontal lobe, which picking up will be impossible if the tension is increased, and a very loose dura indicates atrophy of the brain. The thickness of the dura may be measured approximately by inspection; if normal, the outlines of the convolutions and cerebral vessels may be seen through it. Examine the superior longitudinal sinus before removing the dura. To remove the dura, introduce the amputating knife just

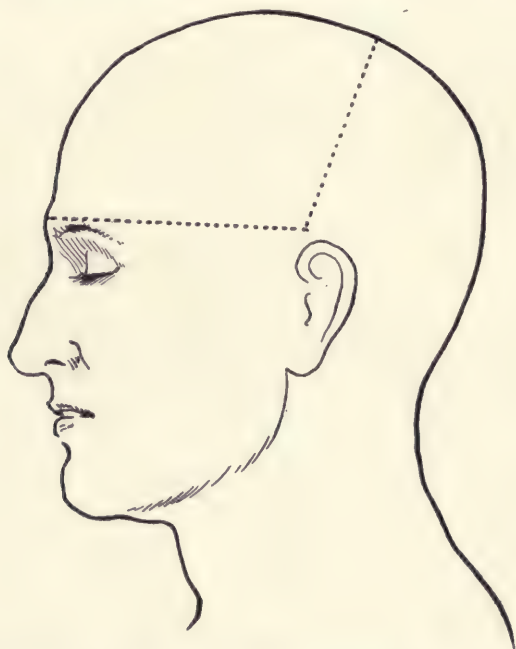


Fig. 36.—Removing the skull cap. The angular method offers many advantages over the original circular method.

to the left of the anterior falx and cut with a sawing motion. Put the fingers under the dura and separate off the vessels; then cut the anterior falx and strip back the dura.

Removing the Brain.—Place the forefinger of the left hand under the frontal lobes and pull upward. With a sharp scalpel cut each pair of cranial nerves near the exit from the cranial cavity. Cut the internal carotid artery and the tentorium with the point of the knife sliding along on the crest of the petrous bone, lifting up the temporal lobe on either side as this is done. Insert the knife

through the foramen magnum, severing the spinal cord and vertebral vessels. The brain is laid on the table.

Examination of the Brain.—The upper surface of the brain is examined and then turned over. Carefully examine each vessel in the circle of Willis. To open the lateral ventricles, strip away the meninges from the top, but leave those on the sides in position in order to support the brain substance. With fingers separate the hemispheres until the corpus callosum comes into view. Each of the lateral ventricles may be opened by inserting the knife without injuring the convolutions. To open the third ventricle, cut the anterior attachment of the corpus callosum and the lateral peduncles, and inspect the interior. To open the fourth ventricle, make a longitudinal incision through the vermis. To examine the cerebellum, make pig-pen incisions into its substance. To examine the cerebral substance, make two sweeping longitudinal incisions of cortex and transverse cuts entirely through the pons (pons varolli).

Examination of Special Sense Organs.—Post-mortem examinations of the eye and ear are rarely made save by experts. After removing the brain, the roof of the orbit or of the middle and internal ears may be chiseled off and these regions examined from above.

Chief Incision of the Trunk.—This extends from Adam's apple (pomum Adami) to the root of the penis (Fig. 37), and is best made with a scalpel or cartilage knife. The skin over the neck is loose, and must be stretched with the fingers of the left hand as the incision proceeds. On reaching the sternum, the belly rather than the point of the knife is used, and the cut made deep to the bone. At the ensiform cartilage the pressure must be diminished. On reaching the epigastrium, a cut is made entirely through to the peritoneum and two fingers of the left hand inserted, one on either side of the knife, the fingers closely following the path of the knife, keeping the tissues taut. The incision is passed around to the left of the umbilicus, so that the connections of the latter with the liver may not be disturbed. On reaching the pubis the cut is made deep to the bone.

Preliminary Abdominal Inspection.—At this time a brief inspection of the abdominal cavity may be made, noting the appearance of the peritoneum and omentum, amount and character of fluid, position of viscera, distention of gut, and perforations. Take

the height of the diaphragm. A transverse abdominal incision may be avoided by running the knife under either rectus and cutting upward to the skin, entirely severing these muscles. If two men are at work, one may continue the examination of the ab-

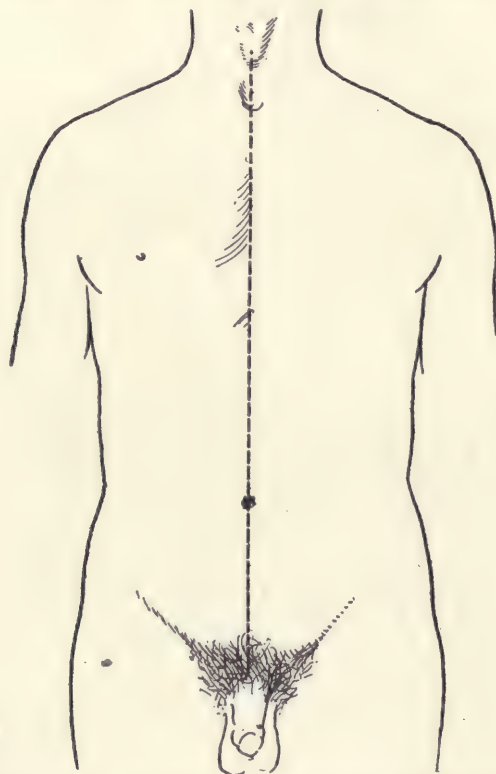


Fig. 37.—Initial incision. The initial incision of the trunk extends from Adam's apple to the root of the penis.

dominal contents, or otherwise a wet cloth should cover these until the thoracic examination has been completed.

Opening the Chest.—At this time examine the breast by a cut underneath, which may be easily hidden. With well-directed cuts peel both flaps of skin and muscle from the ribs as far back as the axilla, examining the glands of this region and also the sternum and ribs. Beginning at the second rib, cut each costal cartilage at its junction with the bony portion of the rib. Cut the diaphragmatic attachments below, and raise the lower end of the

sternum with the cartilages. With the cartilage knife disarticulate the clavicles and first ribs from the sternum. Forcefully separate the tissues of the anterior mediastinum and remove the piece. Avoid cutting the jugulars, as bleeding would be very profuse. Examine the thymus and internal surface of the sternum.

Examination of the Heart.—This is best examined in position.

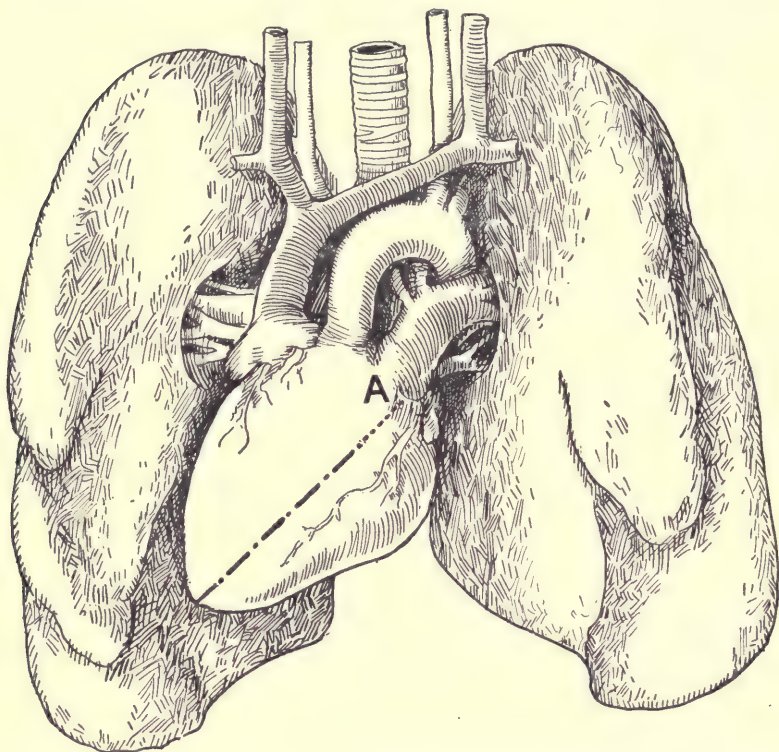


Fig. 38.—Examination of the heart. The heart may be examined in position. A, outlet of left pulmonary veins.

After noting the position of the apex, remove a large portion of the anterior pericardium, and you will be ready to open the left heart. Seize the apex and swing over to the right, holding tautly in this position (Fig. 38), when the left ventricle will become very convex. Run the point of the knife into the apex of the left ventricle, and cut upward in a line ending at the posterior edge of the junction of the left pulmonary vein and left auricle. The actual

cut, however, stops short of the auriculo-ventricular septum. Measure and examine this valve, and then open up the left auricle and pulmonary veins. The valvular openings are usually measured by inserting the fingers. (See page 158.) The aorta is not usually examined at this time. The right heart may then be opened by permitting the organ to return to its natural position, and making a cut through the anterior flap, beginning at its edge, and, perpendicular to the cut, extending it toward the right. The cut should pass through the greatest transverse convexity, and may be continued several inches. Another cut extends upward from this to the auriculo-ventricular septum, and, after measuring and inspecting this valve, the auricle and pulmonary artery are examined. The aorta is measured, and then opened and examined.

Removal and Examination of the Lungs.—Adhesions are loosened between the visceral and parietal pleuræ if possible, as otherwise the parietal layer may be stripped from the ribs. After cutting the bronchial and vascular attachments, both lungs are removed and laid on the table for examination. After a thorough inspection of the pleuræ, numerous incisions will expose the lung substance. Special attention should be given to the apices. Open up the larger bronchi and vessels.

Neck Organs.—These are usually examined in position, and rarely offer much in a diagnostic sense. The incision of the thyroid will demonstrate the presence of cysts or tumors.

Examination of the Spleen.—This is seized with both hands, and the phrenosplenic ligaments stretched or detached. It is laid on the edge of the ribs, and the principal incision made from pole to pole. It may thus be examined and returned to its position without cutting the gastrosplenic omentum.

Removal and Examination of the Kidneys and Adrenals.—Pull over the omentum and intestines to the right. Nick peritoneum between descending colon and spinal column, and then lay aside the knife, completing with the hands a peritoneal dissection of the left kidney and loosening it from its anchorage. Cut the vessels on the bodies of the vertebræ, but cut the ureter somewhat lower down. The kidney is then removed. Now hold it in the palm of the hand in such a manner that the ureter hangs between the fingers, and cut through the opposite convex surface from pole to pole and into the pelvis. Slit the ureters. Attempt to strip the capsule, and make other necessary observations. The right kidney

is removed in a similar manner, and its adrenal is most likely to be closely bound to the liver.

Removal and Examination of the Intestines.—The duodenum need not be removed. Separate the stomach and transverse colon with the fingers, and then cut transverse mesocolon just back of transverse colon, working from below. Tear down the sigmoid mesocolon. Pass a couple of ligatures around the gut at the junction of the sigmoid and rectum, and sever it between these. The hepatic flexure may be torn loose and the ascending colon freed from its attachments to the ileum. The small intestine is next separated from its mesentery by holding it taut and using the knife with a fiddle-bow motion. Pass two ligatures around the junction of the duodenum and the jejunum, and divide the gut between these. The intestines are now floated in a bucket of cold water, opened, washed, and examined. They may be opened with shears having one probe point, or with a pair of ordinary shears having one point protected with a lead buckshot.

Examination of the Duodenum.—This is usually examined in the body of the cadaver, being easily opened with curved shears, carefully avoiding the bile papilla. Squeeze the gall-bladder for evidences of stenosis of its duct; if this is open, the bile should enter the duodenum. A finger may be inserted through the pyloric orifice to examine for patency.

Examination of the Stomach.—It is rarely necessary to remove this organ in order to examine it. A ligature may be passed around the lowest part of the esophagus and the highest part of the duodenum, and a longitudinal incision along the anterior wall midway between the curvatures will expose all parts of the mucosa.

Examination of the Pancreas.—Do not remove it from the cadaver. This is the hardest organ in the body, and a few transverse cuts should be sufficient. A piece may be removed for microscopical study.

Examination of the Liver.—This may be examined in the body, a long incision in the anterior aspect usually sufficing to show all macroscopic morbid changes.

Examination of Retroperitoneal Structures.—Glands, ganglia, vessels, etc., may be examined after removing the pancreas and mesentery.

Removal and Examination of the Male Genitalia.—Separate

the bladder from the anterior abdominal wall with the fingers and an occasional use of the knife. Work the hand in behind and under the rectum, prostate, and urethra, and cut off the mass, taking care to cut the rectum low and the urethra just anterior to the prostate. These organs are laid on the table, opened, and examined.

Removal and Examination of the Female Genitalia.—Separate the bladder from the pubis as in the male, also dissecting loose the rectum from the sacrum with the hand. Separate the cadaver's legs and pass two elliptical cuts around the external genitalia, following the pelvic outlet, meeting in front at the lowest point of the chief incision of the trunk and behind at the back of the anus. Dissect upward, keeping the point of the knife close to the bone. The mass is removed through the superior strait, laid on the table, and the various organs opened and examined.

Removing the Cord.—In case the examinations of the thoracic and abdominal viscera (page 164) are omitted, the cord may be removed from the dorsum, as otherwise the ventral method is preferable.

Removing the Cord Ventralward.—In case this is to be done, remove all thoracic and abdominal organs, and sponge the cavity dry. Beginning at the neck, remove all muscles and soft parts from the bone. Place a block under the lumbar region, and with a heavy scalpel or cartilage knife cut through the last two intervertebral disks, using the belly and not the point of the blade, as transverse processes protect the cord here. With the bone forceps snap the pedicles of the last lumbar vertebra and remove the freed body. Cut loose the other pedicles with a hammer and chisel, working from below upward, cutting disks and removing bodies of the vertebræ as loosened. The spinal roots are put on a tension and cut with scissors, when the cord is lifted out.

Removing the Cord from the Dorsum.—This method is selected in case the remainder of the autopsy is omitted, but, on account of the hypostasis usually present, it is by no means a clean operation. A median incision extends from the hair line to the sacral dimple and marks the tips of the spinous processes. The skin flaps are then dissected back on both sides. The muscle flaps are made by incisions hugging the sides of the spinous processes and dissected in a similar manner. These may be reflected outward, and held in place by hooks, or may be entirely removed. The laminæ and

spinous processes are removed with a saw or chisel, or both, and the cord is released as described above.

Autopsy of the New Born.—Several alterations in technic are necessary when making the necropsy of an infant. The scalp incision is identical with that used in the adult, but the bones may be separated by cutting their membranous connections with shears. Ligatures are passed around the trachea and esophagus before opening the chest. The lungs and the heart should float if the child has breathed. If any lobe of the lung floats, the infant has at least gasped. The stomach is opened under water. Bubbles of gas usually indicate that air has been swallowed, though they are sometimes due to fermentation.

Completing the Autopsy.—Although notes may be taken, it is best to defer the extended discussion of findings. Bones may be wired back into place and incisions carefully sutured. Every effort should be made to restore the cadaver to its best condition, which applies especially to those parts which may be seen at the funeral.

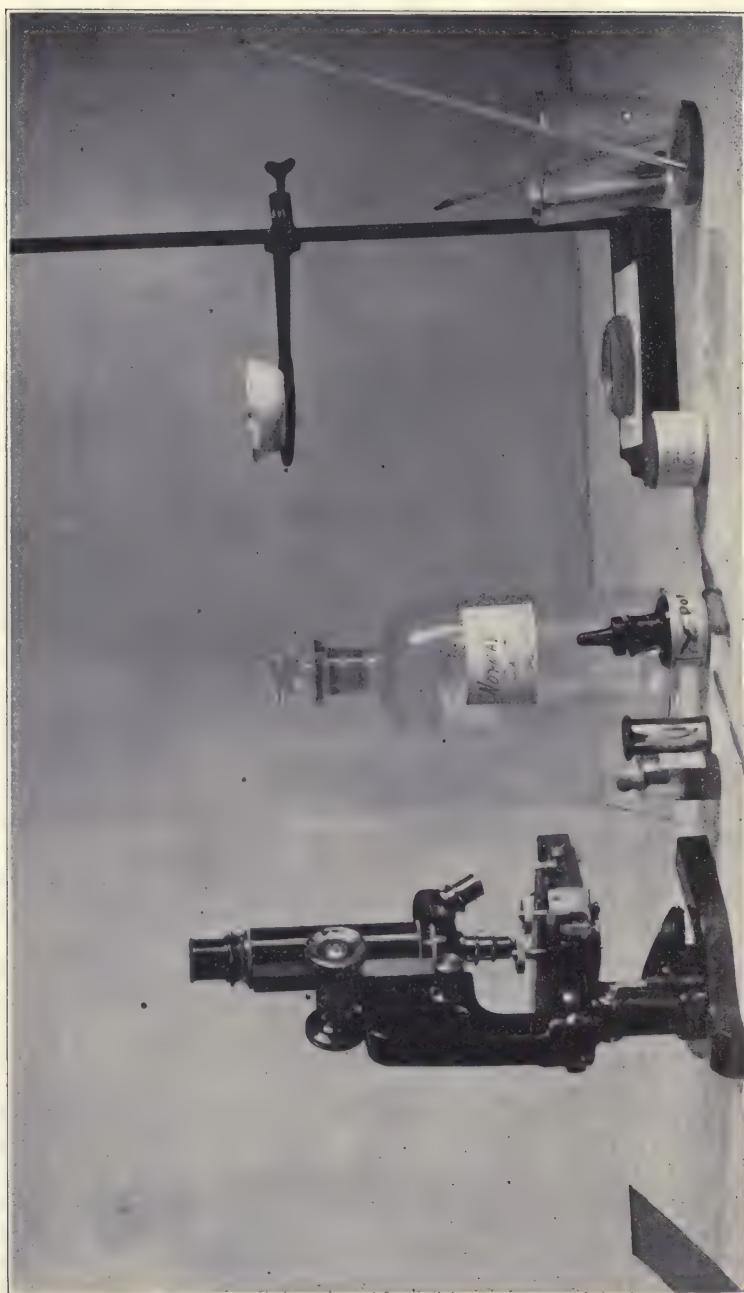


Fig. 39.—Apparatus for finding *treponema pallidum*.

CHAPTER XV.

TO FIND THE TREPONEMA PALLIDUM IN SIX MINUTES.

Apparatus.—

- | | |
|--------------------------------|-------------------------------|
| 1. Beaker or evaporating dish. | 6. Needle. |
| 2. Flame. | 7. Physiologic salt solution. |
| 3. Higgins' carbon suspension. | 8. Scalpel. |
| 4. Medicine dropper. | 9. Slides and cover glasses. |
| 5. Microscope and accessories. | 10. Thermometer. |

Obtaining the Specimen.—Tease lightly the surface of the suspected mucous patch or cutaneous ulcer, or scrape gently with a sharp scalpel the papule or chancre. If the surface of the suspected syphilelcus seems moist or gummy, this first step may be omitted. The fluid, a warm physiologic salt solution, is quickly dropped from the capillary pipette on this surface and as quickly withdrawn (Fig. 40). An ordinary medicine dropper may be drawn out in the flame to serve as a pipette. Four droplets, or less, of the solution usually suffice, as the suspension of the treponemæ will be more concentrated than where larger amounts of the liquid are used. As the solution cools very quickly, there should be no delay. For practical purposes, a physiologic salt solution may be prepared by adding 1 teaspoonful of pure sodium chlorid to 1 quart of distilled water, which should be kept warm, but not hot.

Preparing the Specimen.—A clean slide, on which has been placed a droplet—not a drop—of Higgins' waterproof black drawing ink, is ready to receive 1 or 2 droplets of the suspension as prepared above. After the ink and droplets are thoroughly mixed with a needle or platinum wire, the preparation is ready for examination. A cover glass may be added at once and the specimen

References.—Burri: Wiener Klinische Wochenschrift, July 1, 1909; Williams: Archives of Diagnosis, January, 1910, and Journal of American Medical Association, December 10, 1910; Barach: Journal of American Medical Association, November 26, 1910.

examined wet. This is not, however, the method preferred in all cases by the authors, and, although desiccation often, but not invariably, causes a loss of characteristic windings, a dried preparation is more likely to show a typical ideal field. If a dry examina-



Fig. 40.—Making the suspension.

tion is desired, no cover glass is to be applied until all evidences of moisture are lost. A slight spreading of the droplet is advisable, but this should not be overdone, as otherwise the ideal fields will be difficult to find (Fig. 41). When the preparation has dried,

it will show a black center and a small brown peripheral margin. The former represents carbon deposits, and the latter consists mainly of dried fluid, which contains a very concentrated collection



Fig. 41.—Spreading the mixture. A, correct spread, lighter areas showing usual locations of ideal fields, the bulk of the carbon remaining in the center as the drying proceeds; B, droplet spread too thoroughly, with no concentration of the treponemæ.

of the treponemæ. That portion of the field last to dry contains the most of these germs.

Examination in the Wet.—By this method the examination may be done at once. The treponemæ may be observed in motion, and the characteristic windings or turns of the germs are not lost. The examination is done in a strongly transmitted light, and large masses of carbon are to be avoided when searching the field. The pearly white, almost transparent, treponemæ are easily recognized on a homogeneous brown or black background, and appear highly magnified as a result of this differentiation.

Examination in the Dry.—When all evidences of moisture have disappeared, add a drop of balsam and a clean cover glass. Search for an ideal field; i. e., that area which contains the least carbon and the most organic matter—not only treponemæ, but leukocytes, bacilli, etc. This area is usually located at the periphery if the spreading has not been overdone. The specific microörganism appears much the same as in the wet preparations, except that no motions are observed, and the characteristic windings are sometimes, though not invariably, lost.

Identification of the Treponema Pallidum.—In order to make a positive diagnosis, both wet and dry specimens should be examined. The treponema pallidum may be distinguished from spirochetæ, spirilla, cracks, extraneous fibers, etc., by the following gen-

eral characteristics: a motile corkscrew, much more delicate than the other forms; these turns are close and regular, and appear to be somewhat flexible; its geometric regularity in the spirals, which are deeply cut. A thorough study of this microörganism may be made either from the literature or from control specimens before making a decision.

The scientific world relies almost entirely on the morphological characteristics to identify the treponema, no practical cultural methods being known. If any germ or element is found to be identical in form and motion with Schaudinn's specific microörganism, we are then ready to cast aside as useless not only the ink methods, but the dark field differentiation method on which the expert relies. Nor can the staining time and characteristics be termed constants, for in case the treponema pallidum is actually found in these inks—and it never has been—the drawing inks may then be abolished along with the public drinking cup. (See criticism of the method below.)

Sources of Error.—

1. Use no disinfectant on the syphilecus for several hours before obtaining the specimen. Inquire about the recent use of mercury or 606, as the authors have noted that either may cause the rapid disappearance of the treponemæ from the lesion.
2. Keep the salt solution warm, but not hot.
3. Use droplets, not drops.
4. Do not overdo the spreading.
5. Find an ideal field before attempting to identify the treponemæ.
6. Study well the characteristics of this germ before reaching a decision.

Disadvantages of the Method.—

1. Every field is not ideal.¹
2. Inks are not sterile, and may contain many microörganisms. No report has, however, been made that any observer has identified the treponema in these inks.

Criticism of the Method.—The following criticism has been made of this method: Certain wavy fibers and cracks appear in these inks (Fig. 42), and, while most of them would not receive

¹To those who prefer to use the India ink as suggested by Burri, the "Chin-Chin Liquid Pearl," distributed by Bausch & Lomb Optical Company, is recommended.

very serious consideration by the experienced microscopist, the general practitioner is warned against their use.

Alas, the general practitioner! Although he has graduated from our best medical schools, and usually during the era of microscopy, he is hardly given the credit of being able to distinguish a urinary cast from a cotton fiber. No critic, however, has yet said, "After a careful investigation I can not distinguish fibers and cracks from microorganisms." When competent observers can make this statement, then shall we be prepared to lay aside as worthless not only the simple methods, but practically everything that we have thus far learned about Schaudinn's specific germ. The treponema has not as yet, however, filled all the requirements of Koch's law.

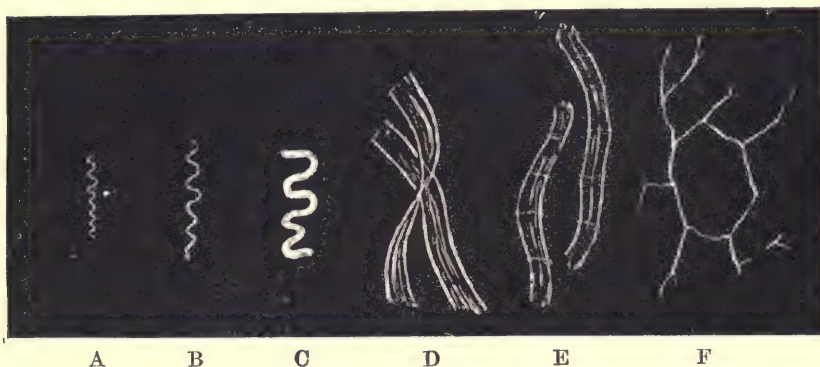


Fig. 42.—*Treponema pallidum*. A, *treponema pallidum*; B, *spirocheta refringens*; C, a spirillum; D, cotton fibers; E, flaxen fibers; F, cracks in ink.

Advantages of the Method.—It is simple, rapid, and the suspension of the treponemæ is twice concentrated, giving in the ideal field a maximum number of germs. For the office or bedside diagnosis of syphilis the practitioner who has mastered his laboratory courses, and who has kept informed on the *treponema pallidum*, may, with a little additional study and practice, depend on the method given in this chapter.

Principle Involved in the Technic.—

1. There are many treponemæ on the surface of every syphileus.
2. These treponemæ show a preference for liquids, having a tendency to leave their host with the drawing off of certain solutions properly applied.

3. Higgins' ink, being a suspension of carbon in a dark fluid, permits, when drying, the formation of the ideal field, which contains most of the treponemæ and but little carbon.

4. The treponemæ are not stained by this ink, but appear as pearly, almost transparent, bodies on a homogeneous brown or black background, and appear magnified as a result of this differentiation.

CHAPTER XVI.

LABORATORY PROPHYLAXIS.

The Safe Operator.—The practice of medicine—no less its workshop—is no place for the diffident practitioner. The days of “miasms” have long passed, and we know too much concerning the subject of bacteriology to hesitate to seek a bit further when opportunity offers. We should not, however, despise the “insignificant little germs.” The competent bacteriologist has usually passed through at least three stages in his development. In the first stage he enters the field trembling with fear, and expects disaster from many sources. He is therefore overcautious, and consequently nothing goes amiss. After a time, in the second stage, he wearies of “details,” his fear of microörganisms grows less and less, and he often explains his carelessness by “vital resistance.” His evolution to the third stage may be delayed for years, but usually takes place when he least expects it. There comes a day of awakening, which may end his bacteriological career, but which usually ushers in the second reign of caution, and he is now qualified to become an expert in this field—in other words, he has become a safe operator.

Germicides, Antiseptics, and Disinfectants.—A germicide is an agent which kills germs, an antiseptic prevents or inhibits their development, while a disinfectant may do either or both. A brief study of the subject will show that distinctions are not sharply drawn, but on one point there seems to be a general agreement among all authorities—i. e., most chemical disinfectants which may safely come into direct contact with the epidermis of the living man are likely to possess very weak germicidal properties. Chemical antiseptics are of service only when present, but, when removed, those germs remaining are—so far as strictly antiseptic action has been realized—freed from any restraining influences.

Bacteriophobia Versus Carelessness.—The man who is “germ afraid” should be debarred from the practice of medicine and from the clinical laboratory. On the other hand, the careless diagnos-

tician is most likely to be a careless therapist, and is by no means a safe worker for either department.

Certain precautions were presented in *Searching for Germs*, page 42. These admonitions were selected not only from standard works, but from actual experiences in the authors' laboratories, and are based on the knowledge concerning germs and their habits and life processes. The operator may carry in his mind a picture of what he is actually doing, and endeavor to explain each step, as follows:

1. He heats to incandescence the platinum loop, thereby destroying all germs which may be contaminating it.

2. He permits it to cool, so that it may not quickly destroy the inoculation.

3. He keeps his hand off it, and does not allow it to touch anything, so that recontamination may not occur.

4. He knows that, if it is held in the air, germs may quickly alight on it, and he therefore proceeds quickly with his technic.

5. He touches the point of the wire to the moist colony, and knows that some of the many thousands of germs present must adhere.

6. He removes the wire with the captured germs, studiously avoiding contamination, and proceeds to inoculate the second tube.

If he is timid or careless, his technic falls short at some point, and his inoculation either fails or, unfortunately, is not made in the second tube.

Special Bacteriological Cautions.—General precautions in regard to sterilization and safe technic will be found in *Searching for Germs*, page 39. When working in the bacteriological laboratory all wounds on the hands should be protected with bichlorid of mercury dressings, or work should be discontinued until an aseptic healing occurs. When working with diphtheritic material the body orifices, including the eyes, must be studiously avoided with the fingers, and the hands should be thoroughly scrubbed and rinsed after the examinations are completed. A gargle of an antiseptic nature should be used while working with the culture and for several days afterward. When much of this work is done, a gargle of this character should be kept in the washroom. Special care of the eyes should be taken when working with all genital secretions, as a single gonococcus in a case of old gleet or leucorrhea may cause an acute and destructive ophthalmia. After searching for

the *treponema pallidum* the hands should be soaked in a 1:1,000 solution of bichlorid of mercury—perhaps the only instance where this chemical acts as a germicide. Netting over the window as a precaution against the housefly and general cleanliness will make the bacteriological workshop many times safer than an indifferent use of chemicals—those false securities in mysterious blue solution which rarely kill germs. Plenty of light should fall on the laboratory table. Do not smoke or eat in a bacteriological laboratory.

Autopsy Prophylaxis.—The routine use of gowns, rubber gloves, and general bacteriological measures is, of course, recommended. The cold water used during the post-mortem may contain bichlorid of mercury, but from this alone safety can not be expected.

Not only are the acute infections—as diphtheria and typhoid—dangerous, but, strangely enough, tuberculosis and actinomycosis seem especially prone to attack the prosector.

Wounds from bone splinters are invariably fatal unless properly treated. The vitality of germs powerful enough to survive the bactericidal action of the bone would indicate also their great virulence when coming in contact with living tissues of a weaker class. Cases of malignant endocarditis are especially dangerous, and the necropsy of such cases should be attended with great care.

Skin tubercles, or the *verrucae necrogenicae*, are common, but are not to be underestimated, as they have a tendency to spread, coalesce, and ulcerate. They should always be cauterized.

Any wound occurring during a necropsy should be made to bleed freely and then cauterized. Bichlorid of mercury dressings should be used, and these should be changed at intervals. No wound, and especially a deep one, should be covered with adhesive plaster or celloidin, as conditions would then become ideal for the *tetanus bacillus* to flourish.

Burns by Fire.—Burns by boiling water, heated apparatus, or even the free flame, are not uncommon in laboratory work. A test tube of water which is being heated should always be pointed away from the operator, and should be continually shaken to avoid “shooting.”

Burns of the first degree should be quickly covered with starch paste or soda water, and afterward smeared with petrolatum and wrapped with cotton or bandages. If as much as one-quarter of the body is affected, the patient should be put to bed immediately, and strychnin as well as morphin administered according to indica-

tions. Such a burn is often followed by a shock or nervous chill, and plenty of bed clothes should be at hand. For the treatment of shock, reference may be made to standard surgical text books. Some fever usually follows a severe burn, for which the treatment should be according to accepted practice.

Burns of the second degree should receive treatment similar to burns of the first degree. The blebs may be punctured with a hypodermic needle, and the serum aspirated or permitted to ooze. The detached epidermis should not, however, be removed at once, but only when it shows signs of putrefaction, as it forms a natural skin graft and supplies epithelial cells to the new skin.

Burns of more severe type are treated by cloths wrung out in sweet oil, petrogen, or other bland oil.

Burns by Chemicals.—Mineral acid burns should be washed immediately with water, which will dilute and remove the acids, which may also be neutralized if soapsuds are applied.

Carbolic acid is not easily removed with water, but alcohol in any of its preparations may be quickly applied, removed, and again applied, as it dissolves, but does not neutralize, the poison. After a thorough washing with alcohol, a piece of gauze soaked in alcohol may be applied three or four times at intervals of half an hour, after which the burn may be treated as if fire, rather than the acid, were the destructive agent.

Burns with alkalis are neutralized with vinegar or other dilute mineral acids, and treated as if they were caused by fire.

Other caustics, as capsicum, may be removed with applications of alcohol.

Explosive Mixtures.—Considerable attention is given to explosive mixtures in the discussion of the properties of the various reagents (page 186), but it may be well to emphasize here some of the more dangerous mixtures of chemicals:

1. Sulphuric acid with water or anything containing water in considerable quantity. **Water should never be added to this acid.** When its dilution is necessary, add the acid carefully drop by drop to the required amount of water.

2. Potassium chlorate, potassium chromate, and potassium permanganate must not be rubbed up with glycerin, sulphur, tannic acid, or other oxidizable substances.

3. A sudden mixing of strong alcoholic solution with nitric acid may cause a quick and severe explosion.

4. Acids must be added slowly to metals and carbonates.
5. Hydrogen forms an explosive mixture with phosphorus and with air.
6. Phosphorus should not be permitted to remain in contact with the air, but must remain under water.
7. Sodium and potassium must be submerged in oil, free from moisture and the air.

Inflammable and Explosive Chemicals.—*Gases*—hydrogen, carbon monoxid. *Volatile liquids*—ether, rhigolene, ethyl chlorid, celloidin solutions. *Nonvolatile liquids*—fixed oils and unguents. *Solids*—phosphorus, urotropin.

Substances Which Should Never be Inhaled.—These include the halogens—i. e., chlorin, bromin, etc.

Arsin must not be inhaled, even in very small quantities, and this precaution applies no less to those gases given off when any preparation of arsenic is burned. Garlic-like fumes are given off by white arsenic when thrown on red-hot coals.

Hydrocyanic acid is poisonous when inhaled in any quantity, even though very dilute, and as the concentration is increased there is likewise an increase in the toxicity, so that the concentrated fumes would instantly kill any person exposed.

The fumes of the strong mineral acids are irritating, and should be avoided.

Hydrogen sulphid and carbon monoxid gases are poisonous, even in moderate quantities.

If chloroform is used in a room lighted by a free flame, a certain amount of chlorin gas is formed, which acts as a corrosive on the respiratory passages.

Sulphuric dioxid and certain of the oxides of nitrogen and carbon dioxid in sufficient quantity are poisonous.

CHAPTER XVII.

INDICATIONS FOR LABORATORY AIDS.

“In which cases shall I resort to the microscope and the test tube?” is a question which often arises in the mind of the physician. It is not the aim of this work to contrast the value of the clinical analysis with other forms of diagnostics, as all these procedures are very important branches of scientific medicine and can not be properly separated. Either overestimating or underestimating laboratory aids is the result of ignorance or confusion, but it is safe to predict that within the next few years this subject will be viewed by the practitioner in a much different light from that of the past.

Below is a list of diseases in which the “mays” and “musts” of laboratory aids are indicated, the black type indicating those cases where expert assistance is considered necessary.

INTERNAL PSOROSPERMIASIS—Examination of excretions for coccidia.

AMEBIC DYSENTERY—Examination of stools for amebæ.

TRYPANOSOMIASIS—This and all other diseases not found in the temperate region have been omitted.

MALARIA—Blood smears are treated with Wright's blood stain and a search made for the plasmodium.

DISTOMIASIS—Examination of excreta for flukes.

TENIA—Examination of stools for segments or ova.

ASCARIS LUMBRICOIDES—Examination of stools for adult worms, searching of muscle bits for encapsulated larvæ, and inspection of blood smears for eosinophilia.

ANKYLOSTOMIASIS—Examination of stools for the characteristic segmented eggs, tests for occult blood,

and studies of blood smears for evidences of eosinophilia.

INTERNAL MYIASIS—Examination of excretions or exudations for maggots.

TYPHOID FEVER—Ehrlich's diazo reaction; blood smears for the study of white cells; simplified macroscopic agglutination test with dead cultures; Russo's methylene blue test; **cultures for typhoid bacilli from rose spots, urine, saliva, and stools; true macroscopic and microscopic Widal tests with living typhoid germs.**

SMALLPOX.

VACCINIA.

VARICELLA.

SCARLET FEVER—Repeated urine examinations as a prognostic measure.

MEASLES.

RUBELLA.

MUMPS.

WHOOPING-COUGH.

INFLUENZA—Search for specific microorganism.

DENGUE.

CEREBROSPINAL FEVER—Lumbar puncture and examination of fluid for causative germ.

LOBAR PNEUMONIA—A routine examination of the sputum is interesting, but rarely conducted as a practical measure, unless there is reason to suspect a tuberculous infection instead of or in addition to the ravages of the pneumococcus.

DIPHTHERIA—Searches for the Klebs-Löffler bacillus.

ERYSIPELAS—Searches for streptococci.

SEPTICEMIA—Blood smears may throw some light on the diagnosis, especially if there is a question in the mind of the therapist whether to use a streptococcus or staphylococcus serum.

SAPREMIA—The germs are usually located at the initial focus, and are rarely present in the blood; they are saprogenic in character, and the culture is usually mixed; it is hardly worth while for the practitioner to attempt to isolate and study these, especially as it would shed but little light on the therapeutic side of the question.

RHEUMATIC FEVER—Searches for the various streptococci; when giving the salicylates in heroic doses, occasional examinations of the urine should be made for the presence of casts and other evidences of poisoning.

YELLOW FEVER.

HYDROPHOBIA—Examination of the nervous system of the dog for the Negri bodies.

TETANUS—A search for the specific

organism may be made along with therapeutic measures, but the latter must never be delayed for an absolute diagnosis.

ACTINOMYCOSIS—Examination of pus for specific microorganisms.

SYPHILIS—Searches for the treponemæ; the various serum reactions; cytological examination of the cerebrospinal fluid in suspected syphilitic meningitis.

GONORRHEA—Searches for the specific coccus.

TUBERCULOSIS—Searches for Koch's specific bacillus and for the evidences of its presence—viz., elastic tissue, lymphocytes, etc., according to which portion of the body is affected; section of suspicious lymph glands and a study of the pathological histology; do not attempt to demonstrate the presence of the specific bacillus in tissues, but try to prove the presence of tubercle formation.

ALCOHOLISM, MORPHINE HABIT, MINERAL POISONS, ETC.—Examinations of foods, drugs, beverages, excretions, etc.; isolation of these poisons in a pure form.

FOOD POISONING—Examination of food samples; by "food poisoning" is commonly meant those disturbances of the vital processes arising from the ingestion of foods in which the poisons or the decomposition products of certain bacteria are present, which field must be reserved for the expert.

SUNSTROKE.

ARTHRITIS DEFORMANS.

CHRONIC RHEUMATISM—If possible, a complete examination of every portion of the body should be made, especially of the tonsils, pleural cavities, and other possible sources of pus; such analyses may be made as are indicated by the findings.

MUSCULAR RHEUMATISM.

GOUT—Examinations of the urine.

DIABETES MELLITUS—Examination of the urine for amount, specific gravity, general appearance, glucose, and diacetic acid; a search of the stools for intestinal parasites.

DIABETES INSIPIDUS—Examination of the urine for amount and specific gravity.

RICKETS—Inquiry into the infant's food; if necessary, an analysis of the mother's milk or cow's milk, and modification accordingly.

OBESITY.

STOMATITIS—Search for the thrush fungus, treponema, etc., as may be indicated; attempt the detection of mercury if such seems to be the cause; determination of reaction of saliva.

DISEASES OF THE SALIVARY GLANDS.

DISEASES OF THE PHARYNX—Ulcers may be examined for pathogenic bacteria, especially the tubercle bacillus, treponema, fusiform bacillus, and spirocheta of Vincent's angina; the diphtheria bacillus and pneumococcus may be found in healthy mouths, and, unless they are present in large numbers, have little significance.

TONSILS—Same as pharynx; the yellow plug of acute tonsillitis is really a colony of germs, and may show thousands of streptococci or staphylococci in almost pure culture.

DISEASES OF THE STOMACH—Unless the diagnosis is evident, it is well to supplement it with an examination of the stomach contents; add to this a test for occult blood should the presence of ulcer or cancer be suspected.

DISEASES OF THE INTESTINE—Proper examination of stools, with limitations in regard to interpreting findings.

APPENDICITIS—A study of the white cells, when properly made, gives valuable diagnostic and prognostic data.

INTESTINAL OBSTRUCTION—Examination of vomited material for evidences of biliary and fecal contamination; macroscopic inspection is often sufficient; indicanuria is said to be present.

JAUNDICE—Examination of the urine for biliary pigments; inspection of stools.

DISEASES OF THE PANCREAS—A Camidge test may be made, but at the present time the inferences drawn from a positive reaction are subject to dispute; tests for urinary glucose.

PERITONITIS—In the chronic forms where ascites is present an examination of the fluid may be made.

ACUTE CORYZA.**HAY FEVER.****EPISTAXIS.**

DISEASES OF THE LARYNX—The symptoms and signs of syphilitic and tuberculous laryngitis are pathognomonic, and the collection of material for microscopic examination is often attended with difficulty.

DISEASES OF THE EAR—A pus may be planted on nutrient agar; a streptococcus or pneumococcus infection is less likely to show a thick and creamy pus in large amounts than where the staphylococcus is the etiological agent.

BRONCHITIS, BRONCHIECTASIS, ASTHMA—Sputum examination.

DISEASES OF THE LUNGS—Sputum examination.

DISEASES OF THE PLEURA—Examination of puncture fluids.

DISEASES OF THE KIDNEY, BLADDER, ETC.—Examination of the urine.

DISEASES OF THE BLOOD—Partial and complete examinations of the blood

for diagnostic and prognostic data; in case of pernicious anemia endeavor to discover a cause, for which examinations of the urine and feces should be made; in case of affection of the gastro-intestinal tract endeavor to locate the cause of chlorosis; in the various forms of leukemia, section and examination of the lymph glands give but little diagnostic data, but where leukemia may be ruled out a study of these glands may aid to differentiate tuberculosis from pseudoleukemia.

STATUS LYMPHATICUS.

ADDISON'S DISEASE.

DISEASES OF THE SPLEEN.

DISEASES OF THE THYROID.

DISEASES OF THE THYMUS.

DISEASES OF THE EYE—In affections of the conjunctiva, associated with exudation, smears may be made of the serum or pus, and these may be fixed, stained, and studied for the etiological element—viz., tubercle bacillus, gonococcus, pneumococcus, Koch-Weeks bacillus, Morax-Axenfeld diplobacillus, streptococcus, diphtheria bacillus, etc.

DISEASES OF THE PERICARDIUM—Considerable experience is necessary when attempting a puncture; the study of the fluid obtained may throw some light on the disease.

ORGANIC DISEASES OF THE HEART—

The sputum may be watched for erythrocytes and heart failure cells; the urine may be examined for casts and albumin.

DISEASES OF THE ARTERIES—Urinalyses.

NEOPLASMS—Section and microscopic examination when bits of suspected tissue can be obtained; when a hidden tumor is suspected, examine the blood for evidences of sarcoma (hemoglobinemia) or carcinoma (cachectic anemia).

PARASITIC SKIN DISEASES—Scrapings from lesions and examinations for parasites; study of the blood smear for eosinophilia.

DISEASES OF THE ALIMENTARY TRACT OF INFANT—Analyses of stools or vomitus; examination of the maternal milk; analysis and proper modification of cow's milk.

UTERINE DISEASES—Study of discharges; section and examination of curettings.

PREGNANCY—Periodical examinations of the urine, with special reference to the quantity of urea; tests for albumose when death of infant is suspected.

DRINKING WATER—Examination for evidences of sewage contamination; detection of lead from water pipes; the more complex problems in sanitation.

CHAPTER XVIII.

GENERAL INFORMATION.

For the convenience of the reader, the first portion of this chapter has been devoted to a study of the physical and chemical properties of certain substances. All stains and reagents described in this book, as well as the more common poisons, are considered and alphabetically arranged for ready reference.



Fig. 43.—Five-inch test tube (enlarged one-third), with various amounts of liquid. In qualitative work approximate amounts are always meant unless otherwise emphasized. A, 5 gtt.; B, 1 cc. or 15 gtt.; C, 1 $\frac{3}{4}$ or 60 gtt.

STAINS, REAGENTS, AND OTHER CHEMICALS.

ACACIA—Gum arabic is soluble in 2 parts of water, forming a thick liquid; insoluble and incompatible with alcohol, ether, oils, mineral acids, ammonia, and tartar emetic.

ACETANILID—Soluble in 2.5 parts of alcohol, but almost insoluble in water; dissolves readily in ether and chloroform; incompatible with chloral, phenol, resorcin, thymol, and aqueous solutions of alkali bromides and iodides.

ACID, ACETIC—Possesses the general properties of all mineral acids.

ACID, CARBOLIC—Is not a mineral acid; possesses a characteristic odor; is freely soluble in glycerin, alcohol, ether, and chloroform; soluble in about 20 parts of water and in 2 parts of sweet oil.

ACID, CHROMIC—Is really not an acid, but chromium trioxid; is soluble in water, but not to be mixed with glycerin or other organic substances, as it explodes; incompatible with alcohol, ether, and nearly all mineral salts; must be kept tightly corked and free from moisture.

ACID, HYDROCHLORIC—A true mineral acid; volatile, and must be kept corked; should be isolated from bottles containing ammonium hydroxid, as the chemical combination of these gases in the air results in the formation of solid ammonium chlorid, spreading a white coating over all laboratory glassware; incompatible with alkalis, silver salts, chlorides, chromates, oxides, permanganates, lead salts, etc.

ACID, HYDROCYANIC—An organic compound, soluble in all ordinary reagents, but used very rarely, as inhalation of its vapor causes instant death.

ACID, NITRIC—A true mineral acid; must not be mixed with alcohol.

ACID, PICRIC—A carboic acid derivative, soluble in 100 parts of water; incompatible with all oxidizable substances, gelatin, albumin, alkaloids, etc.; explosive with sulphur and phosphorus.

ACID, SULPHANILIC—Soluble in warm water, but not in alcohol, etc.; usually purchased ready for the diazo test, but this solution may be prepared by dissolving 1 gram of sulphanilic acid in 50 cc. of concentrated hydrochloric acid and making up the quantity with distilled water to 1,000 cc.

ACID, SULPHURIC—A true mineral acid; water or substances containing water must not be added to it; when necessary to dilute it, add it slowly to the required amount of water, constantly stirring or otherwise mixing.

ALCOHOL, ETHYL—Freely miscible with water, ether, etc.; not to be mixed with nitric acid, as it will explode; incompatible with acacia, albumin, chromic acid, permanganates, etc.; volatile and inflammable.

ALCOHOL, FUSEL (FUSEL OIL)—Mixture of the higher alcohols, especially amyl.

ALCOHOL, METHYL—Volatile and inflammable; poisonous when drunk or even when inhaled in considerable quantity.

ALUM-CARMINE—A nuclear stain, which should be purchased ready for use, but may be made up as follows: to 100 cc. of water add from 3 to 5 percent of ordinary alum and 1 gram of carmine, which is to be boiled fifteen minutes and filtered after cooling, after which the solution is again made up to 100 cc. by the addition of distilled water to replace that lost by boiling and filtering.

AMMONIUM HYDRATE OR AMMONIA HYDROXID—An aqueous solution of ammonia gas; explodes when suddenly mixed with strong

mineral acids; to be kept tightly corked and at some distance from the bottle of hydrochloric acid.

ANTIPYRIN—Easily dissolved in water and the ordinary solvents, but incompatible with the majority of reagents and drugs.

ARSENIC TRIOXID—Soluble in about 5 parts of glycerin; freely soluble in hydrochloric acid, strong alkalies, and alkaline carbonates; very slightly soluble in water and alcohol; incompatible with salts of iron and magnesium and tannic acid.

ATROPIN SULPHATE—Very soluble in water and alcohol.

AZOLITMIN—Purified litmus.

BENZIDIN—Slightly soluble in water; freely soluble in alcohol or ether.

CALCIUM CHLORID—Very soluble in water or alcohol; very deliquescent.

CANADA BALSAM—Should be paper filtered and colorless—not yellow; is not miscible with water or alcohol, but with the volatile oils, chloroform, and ether.

CARBOL-XYLOL—Add 1 part of pure phenol to 3 parts of pure xylol and mix thoroughly.

CARBOL-GENTIAN VIOLET—Should be purchased ready for use in ounce bottles. Some workers prefer to use carbol-fuchsin, which may also be purchased in liquid form ready for use.

CELLOIDIN—Shering's celloidin shreds are sold in ounce bottles, and the substance is usually kept under water until the solutions are needed; it is freely soluble in alcohol and ether, and the solutions are highly inflammable; directions for preparing celloidin solutions will be found in *Essence of Tissue Diagnosis*, page 82.

CHARCOAL—Keep well corked, as it readily absorbs gases; much of the charcoal on the market is already thoroughly saturated and practically valueless.

CHLORAL HYDRATE—Easily dissolved by all the common solvents, but incompatible in alcoholic solutions, and can not be mixed with many other drugs; takes up water easily, and should be kept well stoppered.

CHLOROFORM—Slightly soluble in water, and on account of greater specific gravity forms an under layer; soluble in alcohol, ether, and volatile oils; must be kept in brown bottles and well stoppered, so that its fumes may not come in contact with the free flame, as chlorin is thus rapidly formed.

COCAIN, HYDROCHLORID—Freely soluble in water, alcohol, and chloroform.

COPPER SALTS—Soluble in water and alcohol, usually forming blue solutions.

DIMETHYLAMIDOAZOBENZOL—Should be purchased ready for use in ounce bottles of .5-percent solution.

DISTILLED WATER—Easily obtained at exceedingly low prices from druggists; should be kept well stoppered and in a dry place.

EOSIN, YELLOW—Should be purchased ready for use in ounce bottles; several drops added to a watch glass of distilled water usually give a strong stain.

ETHER—Miscible with alcohol, chloroform, and oils; slightly soluble in water, the excess forming an upper layer; very volatile and inflammable.

ETHYL CHLORID—Usually comes in a tube fitted with a valve for releasing; is highly volatile and inflammable; the tube should be slightly warmed in order to hasten the flow of the gas, but an explosion would quickly follow the application of a high temperature.

FERRIC CHLORID—Soluble in water and alcohol; crystals must be kept well stoppered and in a dry place.

FORMALIN—Formalin is a 40-percent aqueous solution of formaldehyd gas; miscible in alcohol and water in all proportions; forms explosive mixtures, and incompatible with ammonia, alkalies, tannin, gelatin, and salts of iron, copper, and silver; to be kept well stoppered.

GLYCERIN—Miscible in all proportions with water and alcohol; forms explosive mixtures with permanganates and other oxidizing reagents.

GUNZBURG'S REAGENT—A solution of 2 grams of phloroglucin and 1 gram of vanillin in 30 grams of alcohol; should be purchased ready for use in ounce bottles.

HAINES' SOLUTION—A solution of 2 grams of copper sulphate, 20 grams of glycerin, and 9 grams of potassium hydrate in 175 grams of water; test samples by heating—if a reduction takes place and a brown precipitate appears, the reagent is to be cast aside as worthless; to be kept in a dark place.

HAYEM'S SOLUTION—This may be easily prepared by the local druggist as follows: mix together about $\frac{1}{2}$ gram of pure bichlorid of mercury, 5 grams of sodium sulphate, and 1 gram of sodium

chlorid; dissolve in enough distilled water to make 200 cc., and filter if necessary.

HEMALUM—Should be purchased ready for use in ounce bottles.

HYDROGEN DIOXID—Miscible in water and alcohol; incompatible with most chemicals; to be kept cool and quiet.

ODOFORM—Soluble in ether, oils, and boiling water; incompatible with balsam of Peru, tannin, salts of silver and mercury, and certain other chemicals not commonly used.

LEAD ACETATE OR SUGAR OF LEAD—Sparingly soluble in water and alcohol, but extremely soluble in either when heated to boiling; incompatible with many mineral salts.

LÖFFLER'S METHYLENE BLUE—Should be purchased ready for use in ounce bottles; it is a solution of methylene blue to which potassium hydrate has been added.

MARX'S FLUID—See Vascular Dramas, page 68.

MERCURIC CHLORID OR CORROSIVE SUBLIMATE—Fairly soluble in water and alcohol, especially when these have been heated to boiling; incompatible with the salts of many metals, tannic acid, etc.

MERCUROUS CHLORID OR CALOMEL—Practically insoluble in ordinary reagents.

METHYLENE BLUE—Soluble in water; less readily in alcohol; should be purchased ready for use in liquid form in ounce bottles.

METHYL GREEN—Aqueous solution containing 1 percent acetic acid.

MORPHIN SULPHATE—Soluble in water, especially when hot; sparingly soluble in alcohol; incompatible with salts of metals, alkalies, iodides, tannic acid, etc.

NESSLER'S REAGENT—A solution of 10 grams of potassium iodid, 5 grams of mercuric chlorid, and 32 grams of potassium hydrate in distilled water to make 200 cc.

NEUTRAL RED—Soluble in alcohol or water.

OIL SPEARMINT—A true volatile oil.

OPIUM—Soluble in alcohol, which, when in excess, permits the addition of a certain amount of water without precipitation; incompatible with alkalies, silver nitrate, and alkaloidal precipitants.

PHENACETIN—Fairly soluble in alcohol, but hardly in water.

PHENOLPHTHALEIN—Easily soluble in alcohol, but not in water unless small amounts of alkali are added.

PHOSPHORUS—Slightly soluble in alcohol, especially when the

latter is hot, but almost insoluble in water; soluble in the fixed oils and freely soluble in carbon disulphid.

POTASSIUM CHROMATE—Soluble in water, but not in alcohol.

POTASSIUM BICHROMATE OR POTASSIUM DICHROMATE—Soluble in water, especially when the latter is heated; practically insoluble in alcohol.

POTASSIUM HYDRATE—A caustic alkali easily dissolved by water or alcohol.

SILVER NITRATE—Very soluble in water and fairly soluble in alcohol; soluble in 5 parts of distilled water; incompatible with salts of metals, tannic acid series, organic substances, and aqueous solutions of vegetable drugs; keep in brown bottle protected from light.

SODIUM CHLORID—Very soluble in water, but almost insoluble in alcohol; incompatible with alcohol, silver nitrate, and certain salts of lead and mercury.

SODIUM CITRATE—Exceedingly soluble in water; slightly soluble in alcohol.

SODIUM NITRITE—Easily soluble in water; slightly soluble in alcohol; incompatible with many chemicals; to be kept well stoppered.

STRYCHNIN SULPHATE—Fairly soluble in water, especially when the latter is heated or glycerin is added.

STANNOUS CHLORID—Very soluble in water; fairly soluble in alcohol; to be kept well stoppered.

SULPHANOL—Fairly soluble in hot water or alcohol, but sparingly when these solvents are very cold.

SWEET OIL—A true fixed oil, which becomes rancid on exposure to light and air.

THIONIN—Soluble in water, but concentrated solutions usually require filtration at intervals.

TINCTURE TURMERIC—An alcoholic solution of curcuma; to be kept in a dark place.

UROTROPIN OR HEXAMETHYLENAMINE—Soluble in water, but not in other reagents; tablets burn in the air with a hot, colorless flame.

ZINC—This must be chemically pure, or at least arsenic free; should be purchased in the form of short sticks; zinc dust to be used when making up Meyer's blood test solution; to be kept well stoppered. See The Urine in Disease, footnote on page 119.

WRIGHT'S BLOOD STAIN—Should be purchased ready for use in

liquid form in ounce bottles; to be discarded as soon as a precipitate forms; precipitation to be avoided by controlling evaporation; to be kept well stoppered.

WEIGHTS AND MEASURES.

Apothecaries' Weight.

20 grains	=	1 scruple.
3 scruples	=	1 dram.
8 drams	=	1 ounce.
12 ounces	=	1 pound.

Apothecaries' Measure.

60 minims	=	1 fluidram.
8 fluidrams	=	1 fluidounce.
16 fluidounces	=	1 pint.
8 pints	=	1 gallon.

Equivalents.

1 grain	=	60 milligrams.
15 minims	=	1 cubic centimeter.
15 grains	=	1 gram.
1 dram	=	4 grams.
1 fluidram	=	4 cubic centimeters.
1 fluidounce	=	32 cubic centimeters.
1 ounce	=	32 grams.
1 quart	=	1 liter.

A teaspoon contains about $1\frac{1}{3}$ fluidrams, a dessert spoon about $2\frac{1}{2}$ fluidrams, and a tablespoon about 5 fluidrams.

MISCELLANEOUS.

Drop Method for Preparing Approximate Percentage Solutions.

—In qualitative analysis (but not in therapeutics) we may, when considering aqueous solutions, use the terms “drop” and “minim” synonymously, and this method gives fairly accurate percentage solutions. For example, suppose a 10-percent solution of silver nitrate is desired. This substance is soluble in one-half its volume of water, or each drop of a concentrated solution in distilled water contains approximately 2 grains; or, to state it in other terms, to each of these drops must be added 19 drops more of the water to

obtain the desired percentage. This method does not, however, answer for quantitative tests, nor for mixtures intended for therapeutic purposes.

Fahrenheit and Centigrade Equivalents.—On the Fahrenheit thermometer the space between freezing and boiling points is divided into 180 equal parts or degrees, the zero point being 32 degrees below the freezing of water. On the Centigrade thermometer the space between freezing and boiling points is divided into 100 parts or degrees, the zero point being at the freezing of water. A degree of Fahrenheit is therefore $\frac{5}{9}$ degree of Centigrade. The following are simple rules for the conversion of temperature of Fahrenheit to Centigrade and Centigrade to Fahrenheit:

ABOVE 32° F. AND 0° C.

FAHRENHEIT TO CENTIGRADE.—Subtract 32, multiply by 5, divide by 9.

CENTIGRADE TO FAHRENHEIT.—Multiply by 9, divide by 5, add 32.

Fahrenheit to Centigrade.

$$\begin{array}{r} 50^{\circ} \text{ F.} \\ 32 \\ \hline 18 \\ 5 \\ \hline 9)90(10^{\circ} \text{ C.} \end{array}$$

Centigrade to Fahrenheit.

$$\begin{array}{r} 10^{\circ} \text{ C.} \\ 9 \\ \hline 5)90(18 \\ 32 \\ \hline 50^{\circ} \text{ F.} \end{array}$$

The examples show that 50° F. above 0° equals 10 C.° above 0°, and vice versa.

BELOW 32° F. AND 0° C.

FAHRENHEIT TO CENTIGRADE.—Subtract from 32, multiply by 5, divide by 9.

CENTIGRADE TO FAHRENHEIT.—Multiply by 9, divide by 5, subtract from 32.

Fahrenheit to Centigrade.

$$\begin{array}{r} 32 \\ 5^{\circ} \text{ F.} \\ \hline 27 \\ 5 \\ \hline 9)135(15^{\circ} \text{ C.} \end{array}$$

Centigrade to Fahrenheit.

$$\begin{array}{r} 15^{\circ} \text{ C.} \\ 9 \\ \hline 5)135(27 \text{ from } 32 \\ 27 \\ \hline 5^{\circ} \text{ F.} \end{array}$$

The examples show that 5° F. above 0° equal 15° C. below 0°, and vice versa.

Spoon Urinalyses.—These analyses have been popular with the general practitioner as bedside tests, and give fairly good results, but are not to be compared with the urinalyses described in this book (page 111). The following tests are quoted from Richter, as the necessary apparatus may not be at hand, but they would not be accepted by any insurance company nor by any high authority in a consultation.

To test for albumin, take a half teaspoonful of urine, add a small pinch of salt, and heat over a lamp or match. When it begins to steam and bubble, add a few drops of vinegar and watch for precipitate.

To test for glucose, dilute 1 or 2 drops of the urine with a few drops of water in a spoon. Carefully evaporate to dryness with a little heat. Now again slowly heat, when almost suddenly a characteristic orange-brown spot and an unmistakable odor of caramel will prove the presence of sugar. One-third of 1 percent is easily detected in this manner. Urine free from sugar colors a smoky black, and on further heating emits its peculiar urinous odor.

Deodorizers.—Previous references have been made to this subject and certain formulas presented (page 24). The burning of sugar or rags, as practiced by the housewife, is efficient, not only because of its "counter odor," but for the reason that the carbon thus formed in its heated or nascent state readily absorbs obnoxious gases. Foul odors from certain substances may be covered up by certain volatile oils, notably cassia and bergamot.

To Remove Rust from Instruments.—Broder's method will be of value to the surgeon as well as the analyst who desires that his outfit present the best possible appearance. Fill a suitable vessel with a saturated solution of chlorid of tin in distilled water, immerse the rusty instruments, and let them remain over night. Rub dry with chamois after rinsing in running water.

To Remove Stains from Fingers.—Anilin dye stains may be quickly removed from the fingers by washing with a cloth dampened in a solution of 4 parts alcohol and 1 part ether.

Mineral acid stains and those caused by potassium permanganate may require several good scrubblings in hot soapsuds before they disappear.

APPENDIX.

Being a concise report of recent and more special laboratory methods which by virtue of their simplicity and usefulness will appeal as practical to the man who desires to go somewhat more deeply into this enticing department of medicine.

Bedside Estimation of Urinary Acidity.—Reference has been made to the significance and estimation of urinary acidity (pages 109 and 123). To avoid the tedious buret method of titration, Harrower has devised a simple instrument in the form of a graduated test tube of heavy glass. This may be carried in the physician's handbag and the estimations made at the bedside of the patient. The physician has only to fill the tube with the urine up to the 10 cc. mark, add a couple drops of phenolphthalein solution and then the decinormal sodium hydrate solution drop by drop, shaking well after each addition until the first permanent pink is obtained. The percentage is then read off directly from the scale on the side of the tube. By keeping track of the amount of urine passed daily, estimation may be quickly made of the total quantity of acid excreted in that time.

Significance of Indicanuria.—Indican is not a normal constituent of the urine but occurs as a result of proteid decomposition at some point in the body. Thus in the purulent conditions, e. g., hidden abscess, the presence of indican in the urine may lead us to diagnosticate the condition. In malignant growths where a considerable degree of degeneration and necrosis is present, indican may appear in the urine in large amounts; but if we can rule out these two sources of indican, we have still to reckon with another of considerable importance; viz., bacterial decomposition of proteid foodstuffs in the colon. In other words, by the detection of indican in the urine of a person in whom hidden pus or other degenerative changes are unlikely, we gain easily and quickly evidences that affairs in the large bowel are not as they should be, and that it is time that this natural incubator be cleaned out.

The finding of excessive amounts of acid in the urine furnishes further proof, but if such is not present, we are scarcely justified in concluding at once that the indican does not come from the colon. The acids accompanying such a condition may be well cared for by the neutralizing precursors of urea; but nothing is present to render inactive the telltale indican.

While it is not the aim of the authors to delve deeply into the mysteries of indican chemistry, another word must be added to complete the diagnostic and prognostic consideration of this body. **INDICAN IS NOT A NORMAL CONSTITUENT OF THE URINE**, let it be repeated. In case pyogenic processes in the tissues (pyorrhoea alveolaris, otitis media, septic tonsils, sinusitis, tuberculous cavities in the lungs, empyema, gall bladder abscess, chronic appendicitis, salpingitis, pyelitis and chronic gonorrheal prostatitis) neoplasms and true copremia are finally ruled out, we have not yet finished our search for the source of urinary indican. Few of us are entirely normal: a gastric hypoacidity may interfere with the proper digestion of the proteids; or we may be taking too much proteid food. In such cases, however, the indican usually occurs in merest traces, so that a considerable amount of this substance always means a strictly pathological condition. For copremia is not to be regarded lightly, it being quite possible that the poisons absorbed from the bowel are highly toxic to the kidney tissue and may have much to do with the etiology of Bright's disease.

Furthermore, a mere cleaning out of the colon may not cause a disappearance of the urinary indican; but such circumstance does not prove that the condition is not copremia. The same microorganisms quickly proliferate to numbers equal to those first attracting our attention. The bowel must be kept renovated and a new flora (e. g.—the Bulgarian bacillus) introduced. Reduce also the proteid diet, and if these measures fail to reduce the indican, it is very likely that **proteid decomposition in the tissues** is responsible for the indican. This substance occurs in large amounts in enteroptosis inasmuch as the bowel bacilli have ample opportunity to proliferate and decompose the proteids. The same may be said concerning intestinal obstruction or fecal-clogged colon.

Detection of Indican.—The following method has been recommended by Dr. Harrower and others, and is simple as well as

reliable: To one dram of the urine, add one dram of pure hydrochloric acid, one half dram of chloroform and two or three drops of reliable hydrogen peroxide. Shake well for a minute and set aside. If a reaction fails to appear, shake again and set aside. The quantities of liquids used need not be accurate, but should be approximately so.

The chloroform dissolves the indigo which has been liberated by the action of the acid and peroxide; and as it sinks to the bottom of the tube, a blue or violet color is noted, the intensity of the tint varying directly with the amount of indican in the urine. Thus we speak of a trace, a small amount, a moderate amount, a large amount, and a tremendous amount—although we have no very reliable method of estimating the degree of indicanuria. Sometimes a permanent red color may take the place of the blue in the chloroform, and this is called red indican. Red indican probably always comes from the bowel, bearing a relation to skatol not unlike that which indican bears to indol. It is more likely to arise from the decomposition of vegetable proteids in the colon; and such patients are usually irritable whereas those with blue indican are depressed.

Sources of Error.—Inquire concerning the administration of potassium iodid, salicylic acid and urotropin as these may give pseudoreactions. Such drugs should be omitted before testing the urine. Iodid of potash imparts to the chloroform a rose-red color; but this may be driven away by shaking with a few drops of alcohol or sodium hyposulphite, whereas the true red indican is refractory to these reagents.

The urine should be fairly fresh and should contain no preservatives. Often the supernatant liquid becomes of a dark color, but the chloroform does not seem to be able to dissolve this indigo. In such case, add one-half dram of alcohol when upon shaking, solution will be effected by the chloroform-alcohol mixture and the heavy layer comes down blue or violet.

Detection and Significance of Indolacetic Acid.—Many of the poisonous acids formed in the colon and excreted by the kidney, baffle accurate identification by tests applicable in the physician's laboratory. A notable exception is indolacetic acid which may or may not be formed in the copremic process. Its significance is the same as that of indican, though either may be present without traces of the other being found. To test for this substance,

add to about one dram of the urine, one drop of a one percent solution of potassium nitrite and a few drops of hydrochloric acid. If positive, a pink color is noted, which varies in intensity directly with the amount of indolacetic acid present. As soon as the color is or is not noted, the test may be finished for indican with the same sample as directed above.

Significance and Detection of the Bence-Jones Body.—The finding of this substance in the urine may be regarded as pathognomonic of multiple myelomata. Exceptions have been claimed, but these must be very few in number. Acidify the urine if necessary. If it is not clear, filter it. Heat a specimen in a test tube. When it becomes quite warm—almost hot—a marked turbidity or cloudiness may be observed which grows more and more intense as the temperature approaches boiling; but as ebullition begins, the urine begins to clear; and when it boils the cloudiness wholly or nearly disappears. Now if the contents be allowed to cool, the turbidity reappears.

Sulphosalicylic Acid Test for Urinary Albumin.—Physicians are loath to carry nitric acid in their handbags; and in many cases a strong aqueous solution of sulphosalicylic acid may answer equally well. This reagent should not be concentrated, inasmuch as many crystals are deposited in the bottom of the vial and may interfere with the test. It is well to keep the vial tightly stoppered as evaporation appears to encourage the deposition of these crystals.

This is a very sensitive test for serum albumin. It is to be carried out at room temperature—i. e., the urine and the reagent are not to be heated. To one dram of the urine (filtered if not clear), add a couple of drops of the reagent and shake sufficiently to mix. Hold in the light and watch for the appearance of a cloudiness. This usually manifests itself promptly, but may be delayed. In case of question, add a few more drops of the reagent. Rarely more than five drops will be required for a dram of urine. Compare with some of the urine to which the reagent has not been added.

This test may be applied to a clear, unfiltered urine or to one which comes through the filter clear; and this statement applies to most urines, although occasionally a specimen cannot be cleared by filtration. In such contingency, the test is scarcely applicable and we must fall back upon the older methods.

Hermann-Perutz Serum Test for Syphilis.—At present the Wassermann is denied the busy practitioner, as considerable time and experience is necessary for its completion. The Hermann-Perutz reaction was devised to fill this want; and though a new test, it bids fair to find a permanent post in the physician's laboratory. This reaction requires for its completion but three elements, viz.—

1. Reagent A.

Sodium glycocholate	2.0
Cholesterin	0.4
Ninety-five percent. alcohol	100.0

2. Reagent B.

A two-percent aqueous solution of sodium glycocholate.

3. Specimen for examination.

Fresh blood serum from the patient.

The reagents should be prepared just before the test is set up. Some blood from the patient's ear or finger is collected in a sterile test tube and slanted in an icebox over night. (A vacuum bottle with some bits of ice will serve equally well.) In the morning the serum is collected by means of a sterile pipet and placed in a sterile test tube; and a sterile cotton plug is placed in its mouth. The first tube and clot are of no further use in the test. The serum is now "inactivated" by placing in an incubator (or vacuum bottle) at 132–133 degrees F. for thirty minutes. Before the test is set up, dilute Reagent A with sterile distilled water in proportion of 1:20.

In a third small, sterile test tube mix .4c.c. of the serum and .2 cc. of each reagent. These quantities may easily be taken up by the aid of an accurately graduated pipet. (The authors have obtained excellent results with 5 drops of the serum and 3 drops each of the respective reagents; but the original method offers no great difficulties if the physician secure a proper pipet.) The mixture is vigorously shaken and then set aside at room temperature, providing the room does not become chilled or overheated. The tube is examined at frequent intervals. If the reaction is positive, a flocculent precipitate will appear. A control should always be carried out alongside this test, with a serum known to be nonsyphilitic.

Value and Interpretations of the Hermann-Perutz Reaction.—

We believe that the meaning of the positive reaction is identical with that of the Wassermann. If the reaction is positive, the patient has syphilis: if it is negative, we are not certain as to the diagnosis and there is still a possibility that he has syphilis. The disappearance of the reaction under mercury or arsenic is not proof sufficient that the patient is finally cured, although it probably shows some degree of success in this direction. Thus only the positive reaction is of value and **this value is diagnostic only**; and so far as we have conclusive proof is never prognostic. Apparently the reaction is as sensitive as the Wassermann; and is doubtless as dependable. Because of its comparative simplicity, it bids fair for a place among our valuable laboratory methods, not only so far as the needs of the general practitioner are concerned, **but by virtue of this very simplicity, the sources of error are reduced to a minimum.** Carried out in connection with control serums and with the butyric acid test (see page 99) its diagnostic worth is great indeed.

Recent Studies in the Painful Oxalurias.—Several months ago one of the authors in a review of the oxalurias,¹ showed that certain cases of lumbar distress and hematuria may be explained by the mechanical injury to the mucous membrane of the urinary passages, occasioned by the passage of sharp crystals of calcium oxalate; the symptoms increasing in intensity as larger numbers of these crystals are passed. Such a condition may be termed painful oxaluria, **oxaluria dolorosa**, and is by no means an infrequent one, though often missed by the practitioner. The treatment is dietetic and medicinal, never surgical unless other troubles complicate. The practitioner should remember that the crystals of calcium oxalate are very small; and this applies especially to those causing symptoms. They cannot be identified by the low power objectives; and are often missed by the higher powers if the worker is careless. There are other forms of oxaluria, and these are not associated with symptoms; but here the crystals are neither deposited in such large numbers nor so high in the urinary tract; they are smaller and their edges not so sharp and their excretion does not extend over long periods of time. Inasmuch as these crystals are often found in fermenting urines, a fresh sample should be examined before an opinion is ventured.

¹ Medical Record, May 11, 1912.

Dressing for Laboratory Tables.—The following method has appeared several times in the *Journal of the American Medical Association*, and is included in this edition for handy reference. This dressing is practically acid-proof and stain-proof. Considerable care should be used when applying the anilin, as it may be absorbed and is a hemolytic poison. The room should be well ventilated, the worker should use rubber gloves and should not inhale the anilin. The ebonizing solutions are made up as follows:

Ebonizing Solution No. 1:

Copper sulphate	125 gm.
Potassium chlorate	125 gm.
Water	1000 c.c.

Boil until salts are dissolved.

Ebonizing Solution No. 2:

Anilin	120 gm.
Hydrochloric acid	180 c.c.
Water	1000 c.c.

Apply two coats of the first solution while hot, the second as soon as the first has dried. Then two coats of Solution 2 are applied and the wood allowed to dry thoroughly. Next a coat of raw linseed oil is rubbed in by means of a cloth until a nice polish is secured. Finally wash with hot soapsuds. To keep the table in condition, it is necessary now and then to wash with soapsuds and polish with linseed oil. The cost of both solutions will not be over fifty cents and the amounts are sufficient for ten square yards of surface.

Orcein Stain for Fibro-Elastic Tissue.—This is an excellent sputum method. While yellow elastic fibers (coughed-up lung substance) can usually be recognized in unstained sputum examined on a black background by naked eye or with the low power objective and narrowed iris diaphragm, small remnants may be confused. The identification is important, as was shown in the sputum chapter. Unna employs two solutions, the first containing 0.1 grams of orcein, 20 grams of 95-percent alcohol and 5 grams of distilled water. The second solution contains the same amounts of alcohol and distilled water but instead of the orcein, he uses 0.1 gram of concentrated hydrochloric acid. Just before using, equal parts of the two mixtures are combined, and a drop of the stain mixed with the drop of sputum containing the suspected material. Elastic tissue is stained a deep brown.

Salting-Out Sputum Method.—A number of various methods have been devised for concentrating the tubercle bacilli in sputum containing but few of these parasites, thus making their finding more probable. Among these, the best that have been proposed is the "salting-out method" of W. S. Davis. To a quantity of the sputum, is added an equal amount of saturated solution of sodium chloride and the mixture shaken until the mucus is apparently broken up and the liquid homogeneous. The mixture is set at complete rest for at least six hours, and then a sample is taken from the top, by means of a platinum loop, and is smeared and examined.

A Simple Gram Stain.—This was proposed by Dr. L. D. Smith and has given good results in the laboratories of the authors. Six wide-mouthed, 4-ounce bottles are numbered from 1 to 6 and labeled according to the following ingredients: Bottle 1 contains alcohol saturated gentian-violet with distilled water in the proportion of one part stain to three parts water. Bottle 2 contains plain distilled water, Bottle 3 Gram's solution, Bottle 4 absolute methyl alcohol, Bottle 5 plain distilled water and Bottle 6 dilute Ziehl-Neelsen carbol fuchsin (about 1:10).

The procedure is as follows: Dip the fixed smear into Bottle 1 and stir for ten seconds, transfer to Bottle 2 for a few seconds, shake off excess water, then to Bottle 3 for 5 seconds stirring rapidly, thence to Bottle 4 until it destains, stirring rapidly but a few seconds and then to Bottle 5. If destaining is not complete, return to Bottle 4 for a second or so, then to Bottle 5 and finally to Bottle 6. Then wash in tap water. The technic takes about one minute. Paddle each liquid gently but actively with the smeared face of slide.

Invariable Blood Stain.—This method was proposed by one of the authors and appeared in the *Journal of the American Medical Association* (November 1, 1913). The stain has proved so satisfactory and dependable as to have earned the term "invariable." It is especially suited to the needs of the general practitioner especially he who cannot become proficient in technic.

This stain does not fix. Briefly it consists of a nuclear part, a special hematoxylin and a cytoplasmic part, water soluble eosin. In 312 c. c. of a three months' ripened Ehrlich's hematoxylin, 0.1 gram of the eosin is dissolved and the mixture filtered. It is now ready for use, and, if tightly corked, may keep indefinitely, even

improving with age. The mixture will appear to be thick, but if it becomes too thick a few drops of absolute alcohol may be added.

The technic is simple: Merely fix with burning absolute alcohol and after cooling, immerse the preparation in the stain for fifteen minutes. Then wash in tap water and dry. First of all, light a burner (this insures prompt ignition of the alcohol before any of the erythrocytes suffer injury) seize the slide bearing the dried film and, with suitable forceps, hold with film face upward. Quickly cover with absolute alcohol. Shake off excess and then ignite at once in flame, removing preparation immediately from the flame. The alcohol will flash off leaving a dry and perfectly fixed film. Ignition must be prompt and alcohol absolute.

Permit the slide to cool and then place in Coplin jar filled with the "invariable." After fifteen minutes remove the slide and wash in tap water. Dry between blotters or by gentle heat according to standard methods. Staining solution may be used many times.

The method has many advantages, and these will be given briefly, the attention of the reader being called to the original report for particulars:

1. Stain does all that any other blood stain does, so far as every day requirements are concerned.
2. Always can be depended upon. Only one blood film necessary.
3. Simple.
4. Rapid.
5. Nuclear figures especially well stained.
6. Does not overstain.

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